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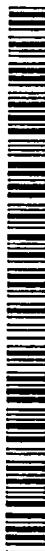
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(54) Title: OPG FUSION PROTEIN COMPOSITIONS AND METHODS

(57) Abstract: The present invention relates to OPG fusion protein compositions, methods of preparation of such compositions and uses thereof. More particularly, the present invention relates to a fusion protein comprising an OPG polypeptide and an immunoglobulin Fc region.

OPG FUSION PROTEIN COMPOSITIONS AND METHODS

Field of the Invention

5 The present invention relates to OPG fusion protein compositions and methods of preparation and use thereof.

Background of the Invention

10 The availability of recombinant proteins for therapeutic use has led to advances in protein modifications in order to enhance or improve the properties of such proteins as pharmaceutical agents. Such modifications can provide enhanced protein 15 protection and decreased degradation by reducing or eliminating proteolysis. Additional advantages include, under certain circumstances, increasing the stability, circulation time, and the biological activity of the therapeutic protein. A review article 20 describing protein modifications is Francis, *Focus on Growth Factors* 3:4-10 (May 1992) (published by Mediscript, London, UK).

One such modification is the use of an Fc region of an immunoglobulin molecule. Antibodies 25 comprise two functionally independent parts, a variable domain known as "Fab", which binds antigen, and a constant domain, known as "Fc" which provides the link to effector functions such as complement or phagocytic cells. The Fc portion of an immunoglobulin has a long 30 plasma half-life, whereas the Fab is short-lived. (Capon, et al., *Nature* 337, 525-531 (1989)).

Therapeutic protein products have been constructed using the Fc domain to provide longer half-life or to incorporate functions such as Fc receptor 35 binding, protein A binding, complement fixation and placental transfer which all reside in the Fc proteins

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of immunoglobulins. *Id.* For example, the Fc region of an IgG1 antibody has been fused to the N-terminal end of CD30 ligand (CD30-L), a molecule which binds CD30 receptors expressed on Hodgkin's Disease tumor cells, 5 anaplastic lymphoma cells, T-cell leukemia cells and other malignant cell types. See, U.S. Patent No. 5,480,981. IL-10, an anti-inflammatory and antirejection agent has been fused to murine Fc γ 2a in order to increase the cytokines short circulating half-10 life. (Zheng et al., *The Journal of Immunology*, 154, 5590-5600 (1995)). Studies have also evaluated the use of tumor necrosis factor receptor linked with the Fc protein of human IgG1 to treat patients with septic shock. (Fisher et al., *N. Engl. J. Med.*, 334: 1697-15 1702 (1996); Van Zee et al., *The Journal of Immunology*, 156: 2221-2230 (1996)). Fc has also been fused with CD4 receptor to produce a therapeutic protein for treatment of AIDS. See, Capon et al., *Nature*, 337:525-531 (1989). In addition, the N-terminus of 20 interleukin-2(IL-2) has also been fused to the Fc portion of IgG1 or IgG3 to overcome the short half life of IL-2 and its systemic toxicity. See, Harvill et al., *Immunotechnology*, 1, 95-105 (1995).

Osteoprotegerin (OPG) has been described in 25 PCT Publication No. WO97/23614 and found to negatively regulate formation of osteoclasts in vitro and in vivo. OPG dramatically increased the bone density in transgenic mice expressing the OPG polypeptide and reduced the extent of bone loss when administered to 30 ovariectomized rats. An analysis of OPG activity in in vitro osteoclast formation revealed that OPG blocks the differentiation of osteoclasts from monocyte/macrophage precursors. OPG appears to have specificity in regulating the extent of osteoclast formation. OPG is 35 a potent factor in blocking bone resorption and may be

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used in the prevention and treatment of loss of bone mass. In vitro and in vivo activity of inhibiting osteoclast formation and blocking loss of bone was also observed in fusion proteins comprising OPG and an Fc domain.

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Fusion of an OPG polypeptide to a heterologous protein or peptide such as an Fc domain may be carried out in a variety of different ways such that the resulting OPG fusion proteins may exhibit 10 variable biological properties and potentially variable effectiveness as therapeutics. For example, an Fc domain may be fused either at the amino terminus or at the carboxy terminus of an OPG polypeptide, it may be fused directly or via a linking molecule, and/or one of 15 the Fc or OPG moieties, or both, may be modified from their native forms. These different OPG fusion protein constructs may show variations in levels of expression, ease of isolation and/or purification, biological activity, and the like.

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Consequently, there exists a need to develop OPG fusion protein compositions as effective therapeutics. Such compositions will exhibit advantageous properties relating to production, isolation, purification, biological activity, 25 stability, and circulation time. The present invention provides such compositions.

Summary of the Invention

The invention provides for OPG fusion protein 30 compositions, methods of preparation of such compositions and uses thereof and provided herein. More particularly, the present invention relates to an OPG fusion protein comprising an OPG protein, or variant, fragment, or derivative thereof, and an Fc 35 protein, or variant, fragment or derivative thereof. Unexpectedly, it has been observed that fusion of an Fc

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region to a truncated OPG polypeptide demonstrates advantages which are not seen in unfused truncated or full-length OPG polypeptides. Such unexpected advantages contribute to lower doses and/or less 5 frequent dosing of the polypeptides of the invention. Thus, as described below in more detail, the present invention has a number of aspects relating to the modification of polypeptides via fusion of an Fc region to an OPG protein (or variants, fragments or 10 derivatives thereof), as well as, specific modifications, preparations and methods of use thereof.

In one aspect, the present invention provides for a protein having a formula selected from the group consisting of: R_1-R_2 , R_2-R_1 , R_1-L-R_2 and R_2-L-R_1 wherein R_1 15 is a Fc protein, or a variant or fragment thereof, R_2 is an OPG protein, or variant or fragment thereof, and L is a linker. The invention also provides for linkers of R_1 and R_2 moieties as described herein.

In another aspect, the present invention 20 provides an OPG fusion protein wherein Fc (or a variant, fragment or derivative thereof) is genetically fused to the carboxy-terminus of an OPG protein (or a variant, fragment or derivative thereof). In another aspect of the invention, an Fc portion may also be 25 linked to the carboxy-terminus of an OPG protein (or a variant, fragment or derivative thereof) by a peptide or chemical linker as known in the art. Additional aspects of the present invention include not only OPG fusion protein compositions, but also nucleic acid 30 sequences encoding such proteins, related vectors and host cells containing such vectors, both useful for producing fusion proteins of the present invention.

In another aspect, the present invention provides for methods of preparing an OPG fusion 35 protein. Using recombinant DNA methods available to one skilled in the art. Chemical methods for the synthesis

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and linking of OPG fusion polypeptides as also provided. Furthermore, such aspects include methods of protein production and purification as well.

5 In another aspect, the present invention provides methods for treating bone disorders, in particular, loss of bone mass. Such bone disorders include osteoporosis, lytic bone diseases resulting from tumor metastasis, hypercalcemia, Paget's disease, bone loss due to rheumatoid arthritis, and the like.

10 In another aspect, the present invention also provides for related pharmaceutical compositions of OPG fusion proteins, variants, fragments and derivatives thereof, for use in the above therapies.

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Description of the Figures

Figure 1 (SEQ ID NO: 1) shows the amino acid sequence of the hinge, CH2 and CH3 regions of human IgG1.

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Figure 2 (SEQ ID NO: 2) shows the amino acid sequence of human OPG [1-401].

25 Figure 3 (SEQ ID NO: 3) shows the amino acid sequence of OPG[22-194]-Fc.

Figure 4 (SEQ ID NO: 4) shows the amino acid sequence of OPG[22-201]-Fc.

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Figure 5 (SEQ ID NO: 5) shows the amino acid sequence of OPG[22-194]-FcΔC.

Figure 6 (SEQ ID NO: 6) shows the amino acid sequence of OPG[22-201]-FcΔC.

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Figure 7 (SEQ ID NO: 7) shows the amino acid sequence of OPG[22-194]-FcG₁₀.

Figure 8 (SEQ ID NO: 8) shows the amino acid sequence of [met]FcΔC-OPG[22-194].

Detailed Description of the Invention

The present invention relates to OPG fusion protein compositions, methods of preparation of such compositions and uses thereof. More particularly, the present invention relates a fusion of an immunoglobulin Fc region to an OPG polypeptide. Unexpectedly, it has been observed that fusion of an Fc region to a truncated OPG polypeptide demonstrates advantages which are not seen with unfused truncated OPG polypeptides or with full-length mature OPG. (wherein full-length mature OPG has 380 amino acids, such as from residues 22 to 401 inclusive, as shown in Figure 2 (SEQ ID NO: 2) It has been further observed that fusion of an Fc region at the carboxy terminus of an OPG polypeptide provides unexpected advantages compared to fusion of an Fc region at the amino terminus of an OPG polypeptide. Accordingly, OPG fusion proteins, and variants, fragments and derivatives thereof, as well as, related methods of use and preparation, are described in more detail below.

The term "OPG" or "OPG polypeptide" refers to a polypeptide comprising the amino acid sequence as set forth in Figure 2 (SEQ ID NO: 2) and related polypeptides described herein. Related polypeptides include allelic variants; splice variants; fragments; derivatives; substitution, deletion, and insertion variants; fusion polypeptides; and non-human homologs. OPG polypeptides may be mature polypeptides, as defined

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herein, and may or may not have an amino terminal methionine residue, depending on the method of preparation.

The term "OPG fusion protein" refers to an OPG protein, or OPG polypeptide which is joined to a heterologous peptide or polypeptide. The OPG fusion proteins of the invention may be prepared by any suitable means known in the art, such as by genetic or chemical fusion of OPG and heterologous peptide or polypeptide moieties. In an embodiment of the invention, the heterologous peptide or polypeptide is an Fc region of an immunoglobulin, preferably a human immunoglobulin. A heterologous peptide or protein may be joined either to the amino terminus or to the carboxy terminus of an OPG polypeptide.

The term "mature OPG polypeptide" or "mature OPG fusion polypeptide" refers to a polypeptide or a fusion polypeptide lacking a leader sequence and may also include other modifications such as proteolytic processing of the amino terminus (with or without a leader sequence) and/or the carboxy terminus, cleavage of a smaller polypeptide from a larger precursor, N-linked and/or O-linked glycosylation, and the like.

The term "Fc" refers to a molecule or sequence comprising the sequence of a non-antigen-binding portion of antibody, whether in monomeric or multimeric form. The original immunoglobulin source of an Fc is preferably of human origin and may be from any isotype, e.g., IgG, IgA, IgM, IgE or IgD. One method of preparation of an isolated Fc molecule involves digestion of an antibody with papain to separate antigen and non-antigen binding portions of the antibody. Another method of preparation of an isolated Fc molecules is production by recombinant DNA expression followed by purification of the Fc molecules so expressed. A full-length Fc consists of the

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following Ig heavy chain regions: C_H1, C_H2 and C_H3 wherein the C_H1 and C_H2 regions are typically connected by a flexible hinge region. In one embodiment, an Fc has the amino acid sequence of IgG₁, such as that shown 5 in Figure 1. The terms "Fc protein", "Fc sequence", "Fc molecules", "Fc region" and "Fc portion" are taken to have the same meaning as "Fc".

The term "fragment" when used in association with Fc or OPG polypeptides, or fusion polypeptides 10 thereof, refers to a peptide or polypeptide that comprises less than the full length amino acid sequence of an Fc or OPG polypeptide. Such a fragment may arise, for example, from a truncation at the amino terminus, a truncation at the carboxy terminus, and/or 15 an internal deletion of a residue(s) from the amino acid sequence. OPG or Fc fragments may result from alternative RNA splicing or from *in vivo* protease activity.

The term "variant" when used in association 20 with Fc or OPG polypeptides, or with fusion polypeptides thereof, refers to a polypeptide comprising an amino acid sequence which contain one or more amino acid sequence substitutions, deletions, and/or additions as compared to native Fc or OPG 25 polypeptide amino acid sequences. Variants may be naturally occurring or artificially constructed. Variants of the invention may be prepared from the corresponding nucleic acid molecules encoding said variants, which have a DNA sequence that varies 30 accordingly from the DNA sequences for native Fc or OPG polypeptides.

The term "derivative" when used in association with Fc or OPG polypeptides, or with fusion polypeptides thereof, refers to Fc or OPG polypeptide 35 variants or fragments thereof, that have been chemically modified, as for example, by covalent

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attachment of one or more polymers, including, but limited to, water soluble polymers, N-linked or O-linked carbohydrates, sugars, phosphates, and/or other such molecules. The derivatives are modified in a 5 manner that is different from native Fc or OPG, either in the type or location of the molecules attached to the polypeptide. Derivatives further includes deletion of one or more chemical groups naturally attached to an Fc or OPG polypeptide.

10 The term "fusion" refers to joining of different peptide or protein segments by genetic or chemical methods wherein the joined ends of the peptide or protein segments may be directly adjacent to each other or may be separated by linker or spacer moieties 15 such as amino acid residues or other linking groups.

Polypeptides

The invention provides for OPG fusion polypeptides and compositions thereof and, more 20 particularly, provides for fusion polypeptides comprising OPG and Fc moieties. Fusions of an Fc region to an OPG polypeptide may be made at the amino terminus of OPG, that is, the carboxy terminus of an Fc region is fused to the amino terminus of OPG. These 25 fusion proteins (and nucleic acids encoding same) are designated herein as FcOPG. It may also be desirable to fuse the carboxy terminus of OPG to the amino terminus of an Fc region. The fusion proteins (and nucleic acids encoding same) are designated herein as 30 OPGFc.

An Fc, or a variant, fragment or derivative thereof, may be from an Ig class. In one embodiment, an Fc is from the IgG class, such as IgG₁, IgG₂, IgG₃, and IgG₄. In another embodiment, an Fc is from IgG1. 35 An Fc may also comprise amino acid residues represented by a combination of any two or more of the Ig classes,

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such as residues from IgG₁ and IgG₂, or from IgG₁, IgG₂ and IgG₃, and so forth. In one embodiment, an Fc region of an OPG fusion protein has the sequence as set forth in Figure 1 (SEQ ID NO: 1) comprising hinge, C_u2 and C_u3 regions of human IgG1. (see Ellison et al., 5 Nucleic Acids Res. 10, 4071-4079 (1982).

In addition to naturally occurring variations in Fc regions, Fc variants, fragments and derivatives may contain non-naturally occurring changes in Fc which 10 are constructed by, for example, introducing substitutions, additions, insertions or deletions of residues or sequences in a native or naturally occurring Fc, or by modifying the Fc portion by chemical modification and the like. In general, Fc 15 variants, fragments and derivatives are prepared such that the increased circulating half-life of Fc fusions to OPG is largely retained.

Also provided by the invention are Fc variants with conservative amino acid substitutions. 20 The term "conservative amino acid substitution" refers to a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. For example, a conservative 25 substitution results from the replacement of a non-polar residue in a polypeptide with any other non-polar residue. General rules for conservative amino acid substitutions are set forth in Table I.

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Table I
Conservative Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val,Leu,Ile	Val
Arg	Lys,Gln,Asn	Lys
Asn	Gln,His,Lys,Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro,Ala	Ala
His	Asn,Gln,Lys,Arg	Arg
Ile	Leu,Val,Met,Ala, Phe,Norleucine	Leu
Leu	Norleucine,Ile, Val,Met,Ala,Phe	Ile
Lys	Arg,Gln,Asn	Arg
Met	Leu,Phe,Ile	Leu
Phe	Leu,Val,Ile,Ala, Tyr	Leu
Pro	Ala	Ala
Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr,Phe	Tyr
Tyr	Trp,Phe,Thr,Ser	Phe
Val	Ile,Met,Leu,Phe, Ala,Norleucine	Leu

5 Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted

10 forms of amino acid moieties. Conservative modifications to the amino acid sequence (and the corresponding modifications to the encoding nucleotides) are expected to produce Fc molecules (and FcOPG fusion proteins) having functional and chemical

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characteristics similar to those of unmodified Fc and FcOPG proteins.

In addition to the substitutions set forth in Table I, any native residue in an Fc region (or in an 5 FcOPG fusion protein) may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis" (Cunningham et al. *Science* **244**, 1081-1085 (1989)).

Substantial modifications in the functional 10 and/or chemical characteristics of an Fc molecule (and an FcOPG fusion protein) may be accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for 15 example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues may be divided into groups based on common side chain properties:

- 20 1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
- 2) neutral hydrophilic: Cys, Ser, Thr;
- 3) acidic: Asp, Glu;
- 4) basic: Asn, Gln, His, Lys, Arg;
- 25 5) residues that influence chain orientation: Gly, Pro; and
- 6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions may involve 30 the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of an Fc or OPG molecule that are homologous with non-human Fc or OPG, or into the non-homologous regions of the molecule.

Cysteine residues in Fc molecules can be 35 deleted or replaced with other amino acids to prevent formation of disulfide crosslinks. In particular, a

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cysteine residue at position 5 of Figure 1 (SEQ. ID. NO. 1) may be substituted with one or more amino acids, such as alanine or serine. Alternatively, the cysteine residue at position 5 could be deleted.

5 An Fc fragment may be prepared by deletion of one or more amino acids at any of positions 1, 2, 3, 4 and 5 as shown in Figure 1 (SEQ ID NO. 1). In one embodiment, the amino acid residues at positions 1-5 inclusive are deleted. Substitutions at these 10 positions can also be made and are within the scope of this invention.

Fc variants may also be made which show reduced binding to Fc receptors which trigger effector functions such as antibody dependent cellular 15 cytotoxicity (ADCC) and activation of complement. Such variants may include leucine at position 20 deleted or substituted with a glutamine residue, glutamate at position 103 deleted or substituted with an alanine residue, and lysines at positions 105 and 107 deleted 20 or substituted with alanine residues (following the numbering as set forth in Figure 1). One or more of such substitutions are contemplated.

In one embodiment, Fc variants will exhibit stronger binding to the FcRn receptor ("salvage receptor") and a longer circulating half-life compared 25 to native Fc. Examples of such variants include amino acid substitutions at one or more of residues 33, 35-42, 59, 72, 75, 77, 95-98, 101, 172-174, 215 and 220-223 as shown in Figure 1 (SEQ ID NO: 1), wherein the 30 substitution(s) confer tighter binding of an Fc variant to the FcRn receptor.

Other Fc variants include one or more tyrosine residues replaced with, for example, phenylalanine residues. In addition, other variant 35 amino acid insertions, deletions and/or substitutions are also contemplated and are within the scope of the

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present invention. Examples include Fc variants disclosed in WO96/32478 and WO97/34630 hereby incorporated by reference. Furthermore, alterations may be in the form of altered amino acids, such as 5 peptidomimetics or D-amino acids.

An Fc protein may be also linked to an OPG protein by "linker" moieties whether chemical or amino acids of varying lengths. Such chemical linkers are well known in the art. Amino acid linker sequences can 10 include but are not limited to:

- (a) ala-ala-ala;
- (b) ala-ala-ala-ala;
- (c) ala-ala-ala-ala-ala;
- (d) gly-gly;
- 15 (e) gly-gly-gly;
- (f) gly-gly-gly-gly-gly;
- (g) gly-gly-gly-gly-gly-gly-gly;
- (h) gly-pro-gly;
- (i) gly-gly-pro-gly-gly;
- 20 (j) val;
- (k) ser-gly-gly-gly-gly-gly-gly-gly-gly;
- gly;
- (l) gly-gly-ser-gly-ser-gly-ala-gly-
- ser-gly-ser-gly-gly-gly-ser-gly-ser-gly-gly;
- 25 (m) a chemical moiety; and
- (n) any combination of subparts (a) through (m).

OPG variants, fragments and derivatives are 30 also provided by the invention and are generally as described hereinabove for Fc molecules, with the exception of the specific locations of the modified amino acid residues. OPG variants, fragments and derivatives are described in PCT WO97/23614 hereby 35 incorporated by reference.

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In a preferred embodiment, the OPG moiety of an OPG fusion protein is a carboxy-terminal truncated form of OPG. Carboxy terminal truncated forms of OPG have one or more amino acids from positions 186-401 in 5 Figure 2 deleted. For example, OPG truncations comprise the amino acid sequence 22-X wherein X is any residue from 185 to 400 inclusive. In another embodiment, OPG truncations comprise the amino acid sequence 22-X wherein X is any residue from 185 to 278 10 inclusive, or from 185 to 293 inclusive, or alternatively, from 194 to 278 inclusive, or from 194 to 293 inclusive. Fusion proteins comprising the OPG truncated polypeptides described herein encompass joining of the OPG and heterologous peptide or 15 polypeptide moieties directly or through a spacer or linker molecule wherein the spacer or linker optionally comprises one or more amino acid residues. Variants and derivatives of the OPG truncated forms described herein are also encompassed by the invention.

20 Preferred fusion proteins of the invention include those wherein the OPG moiety fused to an Fc region comprises the amino acid sequence 22-X wherein X is any residue from positions 194 to 201 inclusive using the numbering as shown in Figure 2 (SEQ ID NO: 25 2). Examples of such fusion proteins include the following:

OPG [22-194]-Fc (Figure 3 and SEQ ID NO: 3)
OPG [22-201]-Fc (Figure 4 and SEQ ID NO: 4)
30 OPG [22-194]-Fc Δ C (Figure 5 and SEQ ID NO: 5)
OPG [22-201]-Fc Δ C (Figure 6 and SEQ ID NO: 6)
OPG [22-194]-FcG₁₀ (Figure 7 and SEQ ID NO: 7)
metFc Δ C-OPG [22-194] (Figure 8 and SEQ ID NO: 8)

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For the preferred polypeptides listed above, the term "Fc" refers to the sequence of human IgG₁ shown in Figure 1 (SEQ ID NO: 1), the term "FcΔC" refers to the sequence shown in Figure 1 (SEQ ID NO: 1) lacking amino acid residues 1-5 inclusive, and the term "FcG₁₀" refers to an Fc moiety lacking amino acid residue 1-9 inclusive and having a ser-(gly)₈ linker.

Nucleic acid molecules

10 Nucleic acid molecules encoding OPG fusion proteins, or variants, fragments or derivatives thereof, are provided for by the invention. Nucleic acid molecules of the invention may be produced using site directed mutagenesis, PCR amplification, or other 15 appropriate methods, where the primer(s) have the desired mutations. See Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory Press, Cold Springs Harbor, N.Y. (1989)), and Ausubel et al. (Current Protocols in Molecular 20 Biology, Wiley and Sons, N.Y. (1994)), for descriptions of mutagenesis techniques. Chemical synthesis using methods described by Engels et al. (Angew. Chem. Intl. Ed. 28, 716-734 (1989)), may also be used to prepare such variants. Other methods known to the skilled 25 artisan may be used as well.

In certain embodiments, nucleic acid variants contain codons which have been altered for optimal expression of an OPG fusion polypeptide in a given host cell. Particular codon alterations will depend upon 30 the OPG fusion polypeptide(s) and host cell(s) selected for expression. Such "codon optimization" can be carried out by a variety of methods, for example, by selecting codons which are preferred for use in highly expressed genes in a given host cell. Computer 35 algorithms which incorporate codon frequency tables

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such as "Ecohigh. Cod" for codon preference of highly expressed bacterial genes may be used and are provided by the University of Wisconsin Package Version 9.0, Genetics Computer Group, Madison, WI. Other useful 5 codon frequency tables include "Celegans_high.cod", "Celegans_low.cod", "Drosophila_high.cod", "Human_high.cod", "Maize_high.cod", and "Yeast_high.cod". In one embodiment, codon 10 optimization may be carried out in either OPG or Fc moieties of the fusion polypeptide.

In another embodiment, nucleic acid molecules encode OPG fusion protein variants with conservative amino acid substitutions as defined hereinabove. For example, conservative amino acid substitutions are made 15 in an OPG and/or in an Fc moiety of a fusion protein. Also provided for are Fc or OPG variants comprising an addition and/or a deletion of one or more N-linked or O-linked glycosylation sites, or comprising Fc or OPG polypeptide fragments as described above. It is 20 understood that nucleic acid molecules of the invention may encode any combination of Fc and/or OPG variants, fragments, and fusion polypeptides described herein.

Vectors and Host cells

25 A nucleic acid molecule encoding an OPG fusion protein is inserted into an appropriate expression vector using standard ligation techniques. The vector is typically selected to be functional in the particular host cell employed (i.e., the vector is 30 compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). A nucleic acid molecule encoding an Fc-OPG protein may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic 35 host cells. Selection of the host cell will depend in part on whether an OPG fusion protein is to be post-

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translationally modified (e.g., glycosylated and/or phosphorylated). If so, yeast, insect, or mammalian host cells are preferable.

Typically, expression vectors used in any of 5 the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the 10 following nucleotides: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a leader sequence for secretion, a ribosome binding site, 15 a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element.

Flanking sequences may be homologous (i.e., from the same species and/or strain as the host cell), 20 heterologous (i.e., from a species other than the host cell species or strain), hybrid (i.e., a combination of flanking sequences from more than one source), synthetic, or native sequences which normally function to regulate OPG and/or Fc protein expression. As such, 25 the source of flanking sequences may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequences is functional in, and can be activated by, the host cell machinery.

30 A leader, or signal, sequence may be used to direct an OPG fusion polypeptide out of the host cell. The signal sequence is most commonly positioned directly at the 5' end of an OPG fusion polypeptide coding region. Many signal sequences have been 35 identified, and any of them that are functional in the selected host cell may be used in conjunction with

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nucleic acid sequences encoding OPG fusion proteins. For example, a signal sequence may be homologous (naturally occurring) or heterologous to an OPG or Fc gene or cDNA. Additionally, a signal sequence may be 5 chemically synthesized using methods set forth above. In most cases, secretion of an OPG fusion polypeptide, and more particularly a fusion of OPG and Fc moieties, from the host cell via the presence of a signal peptide will result in the removal of the signal peptide from 10 the fusion polypeptide.

The signal sequence may be a component of the vector, or it may be a part of a nucleic acid sequence encoding an OPG fusion polypeptide that is inserted into the vector. For example, native OPG DNA encodes a 15 signal sequence at the amino terminus of the protein that is cleaved during post-translational processing of the molecule to form the mature protein. Included within the scope of this invention are OPG nucleotides with the native signal sequence as well as OPG 20 nucleotides wherein the native signal sequence is deleted and replaced with a heterologous signal sequence. A heterologous signal sequence selected should be one that is recognized and processed, i.e., cleaved by a signal peptidase, by the host cell. The 25 invention provides in part for a signal sequence which is the OPG signal sequence as described in WO97/23614. For prokaryotic host cells that do not recognize and process the native OPG signal sequence, the signal sequence is substituted by a prokaryotic signal 30 sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion, the native OPG signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase 35 leaders. In mammalian cell expression the native

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signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

Preferred vectors for practicing this invention are those which are compatible with 5 bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, San Diego, CA), pBSII (Stratagene Company, La Jolla, CA), pET15b (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR α 2 (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

Additional possible vectors include, but are not limited to, cosmids, plasmids or modified viruses, 15 but the vector system must be compatible with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript[®] plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc., La Jolla CA), PCR 20 cloning plasmids designed for cloning Taq-amplified PCR products (e.g., TOPO[™] TA Cloning[®] Kit, PCR2.1[®] plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo 25 Alto, CA). The recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, or other known techniques. After the vector has been constructed and a nucleic acid molecule encoding an OPG polypeptide has been 30 inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression.

Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a 35 yeast cell, an insect cell, or a vertebrate cell). The

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host cell, when cultured under appropriate conditions, synthesizes an OPG polypeptide which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host 5 cell producing it (if it is not secreted). Selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity, such as glycosylation or phosphorylation, and 10 ease of folding into a biologically active molecule.

Suitable host cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC #CCL61 and Urlaub et al., Proc. Natl. Acad. Sci. USA 77, 4216-4220 (1980)), human embryonic kidney 15 (HEK) 293 or 293T cells (ATCC #CRL1573), or 3T3 cells (ATCC #CRL1658). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable 20 mammalian cell lines, are the monkey COS-1 and COS-7 cell lines (ATCC #CRL1651), and the CV-1 cell line (ATCC #CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid 25 cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable 30 mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines. Each of these cell lines is known by and available to those skilled in the art.

35 Similarly useful as host cells suitable for the present invention are bacterial cells. For

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example, the various strains of *E. coli* (e.g., HB101, DH5a, DH10, and MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, 5 *Streptomyces spp.*, and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present 10 invention. Preferred yeast cells include, for example, *Saccharomyces cerevisiae*.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for example in 15 Kitts et al. (Biotechniques, 14, 810-817 (1993)), Lucklow (Curr. Opin. Biotechnol., 4, 564-572 (1993)) and Lucklow et al. (J. Virol., 67, 4566-4579 (1993)). Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

20 Transformation or transfection of an expression vector for an OPG fusion polypeptide into a selected host cell may be accomplished by well known methods including methods such as calcium chloride, electroporation, microinjection, lipofection or the 25 DEAE-dextran method. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook et al., supra.

30 Polypeptide Production

Host cells comprising by transformation or transfection an OPG expression vector may be cultured using standard media well known to the skilled artisan. 35 The media will usually contain all nutrients necessary for the growth and survival of the cells. Suitable

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media for culturing *E. coli* cells are for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells are RPMI 1640, MEM, DMEM, all of which may be supplemented with serum and/or growth factors as required by the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate, and/or fetal calf serum as necessary (Gibco Life Technologies, 5 Gaithersburg, MD).

Typically, an antibiotic or other compound useful for selective growth of transfected or transformed cells is added as a supplement to the media. The compound to be used will be dictated by the 10 selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin; where the selectable marker element 15 is ampicillin resistance, the compound added to the culture medium will be ampicillin.

The amount of an OPG fusion polypeptide produced by a host cell can be evaluated using standard methods known in the art. Such methods include, 20 without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

Where an OPG fusion polypeptide is prepared 25 without a tag attached, and no antibodies are available, other well known procedures for purification can be used. Such procedures include, without limitation, ion exchange chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis 30 in combination with gel elution, and preparative

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isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific). In some cases, two or more of these techniques may be combined to achieve increased purity.

5 If an OPG fusion polypeptide is produced intracellularly, the intracellular material (including inclusion bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, 10 the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

If an OPG fusion polypeptide has formed 15 inclusion bodies in the cytosol, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with 20 chaotropic agent such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion 25 bodies. An OPG polypeptide in its now soluble form can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate an OPG fusion polypeptide, isolation may be accomplished using standard methods such as those set 30 forth below and in Marston et al. (Meth. Enz., 182, 264-275 (1990)).

In some cases, an OPG fusion polypeptide may 35 not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages, can be used to restore biological activity.

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Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices 5 used for inclusion body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In most cases the refolding/oxidation solution will also contain a reducing agent or the 10 reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Some of the commonly used redox couples include cysteine/cystamine, 15 glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol (DTT)/dithiane DTT, and 2-mercaptopropanol (bME)/dithio-b(ME). In many instances, a cosolvent may be used or may be needed to increase the efficiency of the refolding and the more common 20 reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

Derivatives

25 The present OPG fusion proteins, and variants and fragments thereof, are derivatized by attachment of one or more chemical moieties. As an example, a fusion of OPG and Fc polypeptides may be derivatized on either OPG or Fc moieties, or both. These chemically modified 30 derivatives may be further formulated for intraarterial, intraperitoneal, intramuscular subcutaneous, intravenous, oral, nasal, pulmonary, topical or other routes of administration as discussed below. Chemical modification of biologically active 35 proteins has been found to provide additional advantages under certain circumstances, such as

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increasing the stability and circulation time of the therapeutic protein and decreasing immunogenicity. See, U.S. Patent No. 4,179,337. For a review, see Abuchowski *et al.*, in Enzymes as Drugs. (J. S. Holcerberg and J. Roberts, eds. pp. 367-383 (1981)); Francis *et al.*, supra.

The chemical moieties suitable for such derivatization may be selected from among various water soluble polymers. One skilled in the art will be able 10 to select the desired polymer based on such considerations as whether the polymer/protein conjugate will be used therapeutically, and if so, the desired dosage, circulation time, resistance to proteolysis, and other considerations. For the present proteins, 15 the effectiveness of the derivatization may be ascertained by administering the derivative, in the desired form (i.e., by osmotic pump, or, more preferably, by injection or infusion, or, further formulated for oral, pulmonary or nasal delivery, for 20 example), and observing biological effects as described herein.

The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol, copolymers of ethylene glycol/propylene glycol, 25 carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl 30 pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols and polyvinyl alcohol. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in 35 water. Also, succinate and styrene may also be used. In addition, polyaminoacids may be selected from the

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group consisting of serum album (such as human serum albumin), or other polyaminoacids, e.g. lysines.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 5 2 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and 10 manufacturing.

The number of polymer molecules attached to an OPG fusion polypeptide may vary, and one skilled in the art will be able to ascertain the effect on function. One may mono-derivatize, or may provide for 15 a di-, tri-, tetra- or some combination of derivatization, with the same or different chemical moieties (e.g., polymers, such as different weights of polyethylene glycols). The proportion of polymer molecules to protein (or peptide) molecules will vary, 20 as will their concentrations in the reaction mixture. In general, the optimum ratio (in terms of efficiency of reaction in that there is no excess unreacted protein or polymer) will be determined by factors such as the desired degree of derivatization (e.g., mono, 25 di-, tri-, etc.), the molecular weight of the polymer selected, whether the polymer is branched or unbranched, and the reaction conditions.

The chemical moieties should be attached to an OPG fusion protein with consideration of effects on 30 functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art. (EP 0401384 herein incorporated by reference (coupling PEG to G-CSF); Malik et al., Exp. Hematol. 20, 1028-1035 (1992) (reporting pegylation of 35 GM-CSF using tresyl chloride)). For example, polyethylene glycol may be covalently bound through

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amino acid residues having a free amino group (e.g., lysine, arginine or N-terminal residue) or a free carboxyl group (e.g., glutamic acid, aspartic acid, or C-terminal residue). Amino acid residues having a free 5 sulfhydryl group (e.g., cysteine) may also be used. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group. Attachment at residues important for receptor binding should be avoided if receptor binding 10 is desired.

One may specifically desire N-terminally chemically modified OPG fusion protein. Using polyethylene glycol as an example of the chemical moiety, a preparation of substantially N-terminally 15 pegylated OPG fusion polypeptide may be obtained by derivatizing the polypeptide at free amino groups and separating N-terminally pegylated material from a population of pegylated protein molecules.

Alternatively, selective N-terminal chemical 20 modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction 25 conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved. Polyethylene glycol propionaldehyde, containing a single reactive aldehyde, may be used.

30 An N-terminally monopegylated derivative is preferred for ease in production of a therapeutic. N-terminal pegylation ensures a homogenous product as characterization of the product is simplified relative to di-, tri- or other multi-pegylated products. The 35 use of reductive alkylation for preparation of an N-

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terminal product is preferred for ease in commercial manufacturing.

Uses of the Polypeptide

5 The fusion polypeptides of the invention are used in the prevention and/or treatment of loss of bone mass. Bone loss is manifested in a variety of condition including the following:

 Osteoporosis, such as primary osteoporosis,
10 endocrine osteoporosis (hyperthyroidism,
 hyperparathyroidism, Cushing's syndrome, and
 acromegaly), hereditary and congenital forms of
 osteoporosis (osteogenesis imperfecta, homocystinuria,
 Menkes' syndrome, and Riley-Day syndrome) and
15 osteoporosis due to immobilization of extremities;
 Paget's disease of bone (osteitis deformans) in adults
 and juveniles; osteomyelitis, or an infectious lesion
 in bone, leading to bone loss; hypercalcemia resulting
 from solid tumors (breast, lung and kidney) and
20 hematologic malignancies (multiple myeloma, lymphoma and
 leukemia), idiopathic hypercalcemia, and hypercalcemia
 associated with hyperthyroidism, hyperparathyroidism,
 sarcoid, and renal function disorders; osteopenia
 following surgery, induced by steroid administration,
25 and associated with disorders of the small and large
 intestine and with chronic hepatic and renal diseases;
 osteonecrosis, or bone cell death, associated with
 traumatic injury or nontraumatic necrosis associated
 with Gaucher's disease, sickle cell anemia, systemic
30 lupus erythematosus and other conditions; bone loss due
 to rheumatoid arthritis; periodontal bone loss;
 osteolytic metastasis; osteolytic arthritis; and
 prosthetic loosening.

35 In an embodiment of the invention, an OPG fusion polypeptide, by virtue of increased activity and

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circulating half-life, is advantageously used to treat bone loss, and especially bone loss resulting from osteolytic destruction of bone caused by malignant or metastatic tumors. OPG fusion polypeptides of the 5 invention may be used to treat bone loss associated with breast, prostate, thyroid, kidney, lung, esophageal, rectal, bladder, cervical, ovarian and liver cancers as well as cancer of the gastrointestinal tract. Also included is bone loss 10 associated with certain hematological malignancies such as multiple myeloma and lymphomas such as Hodgkin's Disease.

15 Pharmaceutical Compositions

The present invention also provides for pharmaceutical compositions of OPG fusion proteins, and variants, fragments and derivatives thereof. Such pharmaceutical compositions may be for administration 20 for injection, or for oral, pulmonary, nasal, transdermal or other forms of administration. In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of an OPG fusion protein of the invention 25 together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. An effective or a therapeutically effective amount of an OPG fusion protein is an amount sufficient to reduce the amount or rate of bone loss as 30 determined by assays and procedures described below.

Pharmaceutical compositions of the invention include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents 35 (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives

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(e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Hylauronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives.

See, e.g., Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA (1990), pp. 1435-1712, which are herein incorporated by reference. The compositions may be prepared in liquid form, or may be in dried powder, such as lyophilized form. Implantable sustained release formulations are also contemplated, as are transdermal formulations.

Contemplated for use herein are oral solid dosage forms, which are described generally in Remington's Pharmaceutical Sciences, 18th Ed. 1990 (Mack Publishing Co. Easton, PA 18042) at Chapter 89, which is herein incorporated by reference. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Patent No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (e.g., U.S. Patent No. 5,013,556). A description of possible solid dosage forms is given by Marshall, K. In: *Modern Pharmaceutics* Edited by G. S. Bunker and C. T. Rhodes Chapter 10, 1979, herein incorporated by reference. In general, the formulation will include the OPG fusion protein, or a variant, fragment or derivative thereof, and inert ingredients which allow for protection

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against the stomach environment, and release of the biologically active material in the intestine.

An OPG fusion protein may optionally be chemically modified so that oral delivery of the 5 derivative is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the protein (or peptide) molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from 10 the stomach or intestine. Also desired is the increase in overall stability of the protein and increase in circulation time in the body. Examples of such moieties include polyethylene glycol, copolymers of ethylene glycol and propylene glycol, carboxymethyl 15 cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. Abuchowski and Davis, Soluble Polymer-Enzyme Adducts. In: "Enzymes as Drugs", Hocenberg and Roberts, eds., Wiley-Interscience, New York, NY, (1981), pp 367-383; 20 Newmark, et al., J. Appl. Biochem. 4: 185-189 (1982). Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for pharmaceutical usage, as indicated above, are polyethylene glycol moieties.

25 To ensure resistance to degradation in the stomach following oral administration, a coating impermeable to at least pH 5.0 is essential. Examples of the more common inert ingredients that are used as enteric coatings for oral formulations are cellulose 30 acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and Shellac. These 35 coatings may be used as mixed films.

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An OPG fusion protein may be included in a formulation as fine multiparticulates in the form of granules or pellets of particle size about 1 mm. The formulation of the material for capsule administration 5 could also be as a powder, lightly compressed plugs or even as tablets.

Pharmaceutical compositions of the invention include diluents such as carbohydrates, especially mannitol, α -lactose, anhydrous lactose, cellulose, 10 sucrose, modified dextrans and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and 15 Avicell.

Disintegrants may be included in solid dosage formulations. Materials used as disintegrates include but are not limited to starch including the commercial disintegrant based on starch, Explotab. Sodium starch 20 glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange 25 resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

Binders may be used for hard tablets and 30 include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used 35 in alcoholic solutions to granulate the therapeutic.

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Lubricants that may be added to the formulation include, but are not limited to, stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, 5 vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

Glidants that might improve the flow 10 properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

To aid dissolution of an OPG fusion protein 15 composition, a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride 20 or benzethonium chloride. Potential nonionic detergents that could be used as surfactants include lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, 25 sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the protein or derivative either alone or as a mixture in different ratios.

Additives which potentially enhance uptake of 30 a polypeptide are for instance the fatty acids oleic acid, linoleic acid and linolenic acid.

A controlled release formulation may be 35 desirable. An OPG fusion protein may be incorporated into an inert matrix which permits release by either diffusion or leaching mechanisms e.g., gums. Slowly degenerating matrices may also be incorporated into the

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formulation, *e.g.*, alginates, polysaccharides. Another form of a controlled release of this therapeutic is by a method based on the Oros therapeutic system (Alza Corp.), *i.e.*, the drug is enclosed in a semipermeable membrane which allows water to enter and push drug out through a single small opening due to osmotic effects. Some enteric coatings also have a delayed release effect.

Other coatings may be used for the formulation. For example, a film coated tablet may comprise materials from two different groups. The first group includes nonenteric materials such as methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, methylhydroxy-ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl-methyl cellulose, sodium carboxy-methyl cellulose, providone and the polyethylene glycols. The second group consists of the enteric materials that are commonly esters of phthalic acid. A mix of materials might be used to provide the optimum film coating. Film coating may be carried out in a pan coater or in a fluidized bed or by compression coating.

Also contemplated herein is pulmonary delivery of the present protein (or derivatives thereof). The protein (or derivative) is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. (Other reports of this include Adjei *et al.*, *Pharmaceutical Research* 7: 565-569 (1990); Adjei *et al.*, *International Journal of Pharmaceutics* 63: 135-144 (1990) (leuprolide acetate); Braquet *et al.*, *Journal of Cardiovascular Pharmacology* 13 (suppl. 5): s.143-146 (1989) (endothelin-1); Hubbard *et al.*, *Annals of Internal Medicine* 3: 206-212 (1989) (α 1-antitrypsin); Smith *et al.*, *J. Clin. Invest.* 84: 1145-1146 (1989) (α 1-proteinase); Oswein *et al.*, "Aerosolization

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of Proteins", Proceedings of Symposium on Respiratory Drug Delivery II, Keystone, Colorado, March, 1990 (recombinant human growth hormone); Debs et al., The Journal of Immunology 140: 3482-3488 (1988) (interferon 5 γ and tumor necrosis factor α) and U.S. Patent No. 5,284,656 (granulocyte colony stimulating factor).

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic 10 products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

Some specific examples of commercially 15 available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Missouri; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colorado; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, 20 North Carolina; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Massachusetts.

All such devices require the use of 25 formulations suitable for the dispensing of a polypeptide or a polypeptide product. Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to diluents, adjuvants and/or carriers useful in therapy.

An OPG fusion protein (or derivative) should 30 most advantageously be prepared in particulate form with an average particle size of less than 10 μm , most preferably 0.5 to 5 μm , for most effective delivery to the distal lung.

Carriers include carbohydrates such as 35 trehalose, mannitol, xylitol, sucrose, lactose, and

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sorbitol. Other ingredients for use in formulations may include DPPC, DOPE, DSPC and DOPC. Natural or synthetic surfactants may be used. Polyethylene glycol may be used (even apart from its use in derivatizing an 5 OPG fusion protein). Dextrans, such as cyclodextran, may be used. Bile salts and other related enhancers may be used. Cellulose and cellulose derivatives may be used. Amino acids may be used, such as use in a buffer formulation. The use of liposomes, 10 microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

Nasal delivery of an OPG fusion protein is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after 15 administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran. Delivery via transport across other mucus membranes is also 20 contemplated.

Dosages

OPG fusion polypeptides of the invention are administered in a therapeutically effective amount to 25 prevent and/or treat loss of bone associated with metastatic bone disease. A "therapeutically effective amount" of an OPG fusion polypeptide is that amount which reduces the rate and/or extent of loss of bone mass. Bone mass is measured by a variety of known 30 methods such as single photon absorptiometry (SPA), dual photon absorptiometry (DPA), dual energy X-ray absorptiometry (DEXA), quantitative computed tomography (QCT), and ultrasonography (See Johnston et al. in Primer on the Metabolic Bone Disease and Disorders of 35 Mineral Metabolism, 2nd ed., M.J. Favus, ed. Raven Press pp. 137-146). One skilled in the art can use these

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methods to determine a therapeutically effective amount of an OPG fusion polypeptide. A therapeutically effective amount may also be determined by measuring changes in biochemical markers for bone turnover, such 5 as serum osteocalcin, serum alkaline phosphatase, serum procollagen I extension peptides, urinary or serum C-terminal or N-terminal telopeptide of collagen, urinary calcium, hydroxyproline and urinary pyridinoline and deoxypyridinoline. It is generally recognized that a 10 decrease in the levels of the aforementioned biochemical markers indicates that bone resorption is decreased and bone loss is being reduced. Alternatively, a therapeutically effective amount of an OPG fusion polypeptide may also be determined by 15 measuring a change in the mechanical strength of bone, in particular an increase in torsional (twisting) strength of bone.

In general, a therapeutically effective amount of an OPG fusion polypeptide is from about 0.1 20 mg/kg to about 10 mg/kg, preferably from about 1mg/kg to about 10 mg/kg. By virtue of the increased half-life of an OPG fusion polypeptide, especially a fusion of OPG to an immunoglobulin Fc region, the frequency of administration will be less than with unmodified OPG, 25 such as a mature full-length OPG polypeptide. An OPG fusion polypeptide will be administered about one time per month, or alternatively one time every two months, or one time every three months. It will be appreciated that the exact dosage and frequency of administration 30 will depend upon several factors, including formulation, route of administration, condition being treated, and so forth, and may be readily determined by the skilled worker.

The amount of OPG fusion protein which has 35 been administered may be determined using diagnostic assays for the fusion protein. Such diagnostic assays

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may be in the form of an antibody assay, such as an antibody sandwich assay, wherein the antibody specifically binds to an OPG fusion protein but does not bind to endogenous, naturally circulating OPG.

5 Antibody based assays for determining OPG fusion protein levels may be carried out in a variety of formats that are known to one skilled in the art.

10 The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

15

EXAMPLE 1

Construction and Expression of
OPG polypeptides and OPG fusion polypeptides

20

Construction of a recombinant plasmid encoding OPG[1-401] as shown in Figure 2 (SEQ ID NO: 2) is described in WO97/23614 hereby incorporated by reference. This plasmid was used in mammalian host 25 cells to produce a mature full-length OPG polypeptide having amino acid residues 22 to 401 inclusive as shown in Figure 2 (SEQ ID NO: 2). Plasmids encoding OPG[1-201] and OPG[1-201]-Fc polypeptides were constructed generally as described in WO97/23614. These plasmids 30 were used to produce OPG[22-201] and OPG[22-201]-Fc polypeptides.

OPG[1-194] was constructed by PCR using oligonucleotides 1745-92 and 1789-04 and OPG cDNA as a template. The sense primer (1745-92) created an XbaI 35 site for cloning and a consensus Kozak sequence before the initiator ATG. The antisense primer (1789-04)

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placed a stop codon after amino acid residue 194 and a SalI restriction site for cloning. This PCR product was cloned into pDSR α 19 to generate pDSR α 19-huOPG[1-194] for mammalian expression of an OPG[22-194] polypeptide.

OPG [1-293] was constructed by PCR using oligonucleotides 1745-92 and 1745-94 and OPG cDNA as a template. The sense primer (1745-92) created an XbaI site for cloning and a consensus Kozak sequence before the initiator ATG. The antisense primer (1745-94) placed a stop codon after amino acid residue 293 and a SalI restriction site for cloning. This PCR product was cloned into pDSR α 19 to generate pDSR α 19:huOPG[1-293] for mammalian expression of OPG[22-293].

15

1745-92 (SEQ ID NO: 9) 5'-AAG TCTAGA CCACC ATG AAC AAG TTG CTG T-3'

XbaI Kozak OPG coding

20

1745-94 (SEQ ID NO: 10) 5'-GCTA GTCGA CTA CTC GAA GGT GAG GTT AGC AT-3'

SalI * OPG coding

25

1789-04 (SEQ ID NO: 11) 5'-ATCT GTCGA CTA TTT TTG AGT TGA TTC AC-3'

SalI * OPG coding

Construction of OPG[1-194]-Fc Δ C

30

The plasmid pDSR α 19:OPG[1-194]-Fc Δ C was constructed from the plasmid pDSR α 2:OPG[1-201]-Fc using PCR methods to remove an unpaired cysteine at the 3' end of the OPG segment and an unpaired cysteine at the 5' end of the Fc segment. This clone was then used as a template for PCR to obtain the OPG domain. The 5'

35

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OPG primer incorporated an *Xba*I site (TCTAGA) for cloning and a "CCACC" Kozak sequence before the initiator Met codon. The 3' OPG primer incorporated a *Sal*I site (GTCGAC) for cloning the Fc domain. The PCR 5 generated a 592 bp fragment of the OPG gene, encoding the first 194 amino acid residues of the OPG protein. The PCR product was cut with *Xba*I and *Sal*I and cloned into pDSR α 19 to generate the final construct, called plasmid p615.

10

Sense OPG primer (1745-92) (SEQ ID NO: 12):

5'-AAG TCTAGA CCACC ATG AAC AAG TTG CTG T-3'
*Xba*I Site Kozak OPG coding

15

Antisense OPG primer (1775-27) (SEQ ID NO: 13):

5'- CACGC GTCGAC TTT TTG AGT TGA TTC ACT GTT TCC-3'
*Sal*I Site OPG coding

20

The clone pDSR α 2/OPG[1-201]-Fc was used as a template to obtain the Fc domain. The PCR generated the Fc carboxy-terminal 227 aa including the hinge, C_n2 and C_n3 domains. The 5' Fc primer incorporated a *Sal*I site (encoding "VD") and the 3' Fc primer incorporated a *Xho*I site (CTCGAG) after the Fc termination codon. The 25 Fc PCR product was cloned into the *Sal*I site of p615 to yield pDSR α 19:OPG[1-194]-Fc Δ C which produces OPG[22-194]-Fc Δ C upon expression in mammalian cells. The fusion protein contains an extra valine at the Fc-OPG junction. The *Xho*I site is lost in the ligation.

30

Sense Fc primer (1476-25) (SEQ ID NO: 14):

5'- AATCT GTCGAC AAA ACT CAC ACA TGC-3'
*Sal*I Site Fc coding

35

Antisense Fc primer (1504-63) (SEQ ID NO: 15):

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5' - CCATG CTCGAG TTA TCA TTT ACC CGG AGA CAG G-3'
XhoI Site * Fc coding

Construction of OPG[1-194]-FcG10

5 An Fc region with a G10 hinge (one serine and
 eight glycine residues) was constructed by PCR using
 primers 1775-30 and 1504-63 and OPG[1-201]-Fc cDNA as a
 template. The product was subcloned into pCRscript
 (pCRscriptFcG10BspE) and sequenced. OPG[1-194] was
 10 obtained by PCR using primers 1745-92 and 1790-72 and
 OPG[1-201]-Fc cDNA as a template. The PCR product was
 subcloned into pCRScript and sequenced. An Xba/BspEI
 fragment containing OPG[1-194] sequence and a
 BspEI/XhoI fragment containing Fc with a G10 hinge were
 15 then subcloned into pDSR α 19. This plasmid produces
 OPG[22-194]-FcG10 upon expression in mammalian cells.
 The amino acid sequence is shown in Figure 7.

20 G10-Fc 5' Primer: (SEQ ID NO: 16)
 1775-30 5'-AA TCCGGA GGAGGTGGTGGAGGTGGG GGTACC TGCCCACCGTGCG-3'
 BspEI Gly Linker KpnI Fc Domain →
 S G G G G G G G T C P P C

25 G10-Fc 3' Primer: (SEQ ID NO: 17)
 1504-63 5'-CCATG CTCGAG TTA TCA TTT ACC CGG AGA CAG G-3'
 XhoI * * K G P S L

30 OPG 5' Primer: (SEQ ID NO: 18)
 1745-92 5'- AAG TCTAGA CCACC ATG AAC AAG TTG CTG T-3'
 XbaI Kozak opg Coding →
 M N K L L

35 OPG 3' Primer: (SEQ ID NO: 19)
 1790-72 5'- CC TCCGGA TTT TTG AGT TGA TTC ACT GTT TCC AGA-3'
 BspEI K Q T S E S N G S

Construction of Fc Δ C-OPG[22-194]

40 A DNA molecule encoding Fc Δ C-OPG [22-194] was
 generated by standard PCR techniques using the
 pDSR α 2:OPG[1-201]-Fc DNA as a template. The Fc portion

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was generated using oligonucleotides 1757-22 and 1757-23. The 1757-22 primer has an in frame Epo BssHII signal to place the Fc downstream from the erythropoietin signal sequence (the signal sequence is 5 described in U.S. Patent No. 4,703,008). The 1757-23 primer fuses the last amino acid of the Fc domain to amino acid residue 22 of human OPG. The OPG portion was generated using oligonucleotides 1757-24 and 1789-04. The 1789-04 primer places a stop codon after amino 10 acid 194 of human OPG and a SalI site for cloning. These two purified products were then used as a template to generate the Fc/OPG fusion molecule with primers 1757-22 and 1789-04. The resulting PCR product was digested with BssHII and SalI, purified and cloned 15 into BssHII/SalI digested pDSR α 19. Expression of this plasmid in a mammalian host cell produces Fc Δ C-OPG[22-194] as shown in Figure 8 (SEQ ID NO: 8) with the modification that the amino terminal methionine is replaced with the amino acids ala-pro.

20 Sense Fc primer (1757-22): (SEQ ID NO: 20)

5'-TTG GCGCGC CCA AAT CTT GTG ACA AAA CT-3'
BssHII

25 Antisense Fc/OPG primer (1757-23): (SEQ ID NO: 21)

5'-CTT TGG AGG AAA CGT TTC TTT ACC CGG AGA CAG GGA-3'
OPG → | ← Fc

Sense Fc/OPG Primer (1757-24): (SEQ ID NO: 22)

30 5'-TCC CTG TCT CCG GGT AAA GAA ACG TTT CCT CCA AAG-3'
Fc → | ← OPG

Antisense OPG Primer (1789-04): (SEQ ID NO: 23)

5'-ATCT GTCGA CTA TTT TTG AGT TGA TTC AC-3'

35 SalI * OPG Coding

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The vector pDSR α 2 has been described previously (see WO90/14363 and Figure 12 therein, which is incorporated by reference). The vector pDSR α 19 is a 5 modified form of pDSR α 2 which is functionally similar but contains the following changes from pDSR α 2:

- 1) The α FSH polyA was shortened approximately 1400 bp from the 3' end. It is now 885bp and ends at the NdeI site.
- 10 2) The dihydrofolate reductase (DHFR) promoter was shortened from the 5' end by approximately 1 kb and now only contains 209 bp.
- 15 3) An approximately 550bp BglII fragment in the DHFR polyA was deleted.

Conditions for the purification of truncated and fusion polypeptides from conditioned media are generally described in WO97/23614

20 Construction of met Fc Δ c-OPG[22-194]

A met huOPG[22-194] coding sequence was constructed by the following procedure. Synthetic oligonucleotides were constructed consisting of overlapping 50-mers which comprised 25 the entire top and bottom strands of the OPG DNA coding sequence. The internal 50-mer oligos were phosphorylated, annealed, and ligated overnight. The outside oligos, 34-mers, were used in the polymerase chain reaction (PCR) as primers to 30 amplify the full length gene. The PCR reaction was performed using Taq DNA polymerase and additional reaction components as supplied in kit form (Boehringer Mannheim). The resulting 584 base pair PCR product was purified by 1% agarose 35 gel electrophoresis and extracted from the gel

- 45 -

using the QIAquick spin column method (Qiagen). The gel purified fragment was then digested with the restriction enzymes *Xba*I and *Bam*HI (Boehringer Mannheim). A ligation reaction was 5 performed with the fragment described above and the plasmid vector pAMG21 (ATCC accession number 98113) digested with the same restriction enzymes. The ligated DNA was transformed by electroporation into *E. coli* strain #393. Clones 10 were selected for kanamycin antibiotic resistance, plasmid was isolated, and the sequence of the coding region was checked by DNA sequencing. The initial clone selected (referred to as plasmid A) was shown by DNA sequencing to 15 have significant errors in the middle of the gene. The gene sequence was repaired by digesting plasmid A with the restriction enzymes *Spe*I and *Hpa*I and using the resulting product as the vector fragment. A new insert fragment was 20 prepared by PCR of the original ligated oligonucleotide mixture with internal oligonucleotides 1466-91 and 1467-03 as PCR primers in the polymerase chain reaction. The insert fragment was digested with *Spe*I and *Hpa*I 25 and ligated into the plasmid A vector to replace the DNA fragment containing the errors. Transformation, selection, and plasmid isolation were performed as described above. A clone (plasmid B) was confirmed by DNA sequencing as 30 having the correct sequence for human OPG[22-194].

Top strand oligonucleotides 1466-90 to 1467-01:

35 1466-90 (SEQ ID NO: 24):
5' AACAAACTCTAGATTGTTTAACTAATTAAAGG-3'

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1466-91 (SEQ ID NO: 25):

5' AGGAATAACATATGGAAACTTTCCACCTAAATATCTTCATTATGATGAA-3'

5 1466-92 (SEQ ID NO: 26):

5' GAAACTAGTCACCAGCTGCTGTGCGACAAATGTCCTCCGGTACCTACCT-3'

1466-93 (SEQ ID NO: 27):

5' GAAACAGCACTGCACCGCTAAATGGAAAACCGTTGCGCTCCTGTCCGG-3'

10

1466-94 (SEQ ID NO: 28):

5' ACCACTACTACACCGACTCCTGGCACACCTCCGACGAATGCCTGTACTGC-3'

1466-95 (SEQ ID NO: 29):

15 5' TCACCGGTTGCAAGGAGCTGCAGTACGTTAACAGGAATGCAACCGTAC-3'

1466-96 (SEQ ID NO: 30):

5' GCACAACCGTGTGCAATGCAAAGAAGGTCGTTACCTGGAGATCGAAT-3'

20 1466-97 (SEQ ID NO: 31):

5' TCTGCCCTGAAACACCGTTCCCTGTCCGCCTGGTTTCGGTGTACAGGCT-3'

1466-98 (SEQ ID NO: 32):

5' GGTACCCCGGAACGTAACACCGTTGCAAACGTTGCCCGACGGTTCTT-3'

25

1466-99 (SEQ ID NO:33):

5' CTCCAACGAAACCTCGAGCAAAGCTCCGTGCCGTAAACACACCAACTGCT-3'

1467-00 (SEQ ID NO: 34):

30 5' CCGTTTCCGGTCTCCTGTTAACCCAGAAAGGTAACGCTACCCACGACAAC-3'

1467-01 (SEQ ID NO: 35):

5' ATCTGCTCCGGTAACCTCGAGTCGACCCAGAAATAATGGATCCAAACAA-3'

35 Bottom strand oligonucleotides 1476-02 through 1476-13

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1467-02 (SEQ ID NO: 36):

5'-TTGTTTGGGATCCATTATTCTGGTCGACTCGG-3'

1467-03 (SEQ ID NO: 37):

5' AGTTACCGGAGCAGATGTTGTCGTGGTAGCGTTACCTTCTGGGTTAAC-3'

1467-04 (SEQ ID NO: 38):

5' AGGAGACCGAAAACGGAGCAGTTGGTGTGTTACGGCACGGAGCTTGCT-3'

10 1467-05 (SEQ ID NO: 39):

5' CGAGGTTTCGTTGGAGAAGAAACCGTCCGGGCAACGTTGCAAACGGTGT-3'

1467-06 (SEQ ID NO: 40):

5' TACGTTCCGGGGTACCAAGCCTGTACAACACCGAAACCAGGCGGACAGGAA-3'

15

1467-07 (SEQ ID NO: 41):

5' CGGTGTTTCAGGCAGAAATCGATCTCCAGGTAAACGACCTTCTTGCATTTC-3'

1467-08 (SEQ ID NO: 42):

20 5' GCAAACACGGTTGCGTACGGTTGCATTCTGTTAACGTACTGCAGCT-3'

1467-09 (SEQ ID NO: 43):

5' CCTTGCAAACCGGTGAGCAGTACAGGCATTGTCGGAGGTGTGCCAGGAG-3'

25 1467-10 (SEQ ID NO: 44):

5' TCGGTGTAGTAGTGGTCCGGACAAGGAGCGCAAACGGTTTCCATTAGC-3'

1467-11 (SEQ ID NO: 45):

5' GGTGCAGTGCTGTTCAAGGTAGGTACCCGGAGGACATTGTCGCACAGCA-3'

30

1467-12 (SEQ ID NO: 46):

5' GCTGGTGACTIONTTCTCATCATAATGAAGATATTAGGTGGAAAAGTT-3'

1467-13 (SEQ ID NO: 47):

35 5' TCCATATGTTATTCTCCTTAATTAGTTAAAACAAATCTAGAGTTGTT-3'

Fusion of human OPG[22-194] DNA sequence described above to human IgG, Fc Δ C was performed as follows. Plasmid DNA comprising an 5 insert of OPG DNA coding sequence described above fused at its amino terminus to the Fc region of plasmid pFc-A3 was digested with the restriction enzymes *Nde*I and *Spe*I. Plasmid pFc-A3 has been described in WO97/23614. The resulting plasmid 10 vector fragment contained the OPG coding sequence minus the first fourteen codons of the gene (up to the *Spe*I site). This was designated as vector C. The insert was created by performing the polymerase chain reaction using a DNA sequence as 15 shown in SEQ ID NO:13 and SEQ ID NO:14 as set forth in WO98/28427 as the template. A universal 5' primer (#1209-85) for the plasmid pAMG21 (ATCC accession no. 98113) was used to prime the 5' end of the Fc sequence (an *Nde*I site already existed 20 at the beginning of the Fc sequence). Two oligonucleotide primers were designed to prime at the 3' end of the Fc coding sequence while adding an overlap region identical to the 5' end of the osteoprotegerin gene. The first primer, 1595-18, 25 was designed to prime the 3' end of the Fc coding sequence and add the first codons of the 5' end of the osteoprotegerin sequence. A second primer, 1585-16, primed at the 3' end of the previously mentioned primer and added additional 30 OPG coding sequence through the *Spe*I site at codon fourteen. The first round of PCR was performed using a DNA molecule having the sequence in SEQ ID NO:13 and SEQ ID NO:14 of WO98/28427 as template, and primers 1209-85 and 35 1595-18 with Taq polymerase as previously

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described. The 799 base pair PCR product of this reaction was gel purified and used as template in a second PCR reaction with primers 1209-85 and 1585-16. The 825 base pair product of the second 5 PCR reaction was gel purified, digested with *Nde*I and *Spe*I, and ligated into vector C described above. The ligation mixture was transformed into *E. coli* and a clone was isolated and confirmed by DNA sequencing to have the correct OPG coding 10 sequence. The resulting plasmid encodes [met]FcΔC-huOPG [22-194] having the amino acid sequence shown in Figure 8 (SEQ ID NO: 8).

Primer 1209-85: (SEQ ID NO: 48)
15 5'-CGTACAGGTTACGCAAGAAAATGG-3'

Primer 1585-16: (SEQ ID NO: 49)
5'ACAAACACTAGTTCTTCATCATAATGAAGATATTAGGTGGAAACGT
3'
20
Primer 1595-18: (SEQ ID NO: 50)
5'GAAGATATTTAGGTGGAACGTTCTTACCCGGAGACAGGGAG-3'

25 Expression of a DNA sequence encoding [met]FcΔC-huOPG[22-194] in pAMG21 was performed generally as described in WO97/23614. The fusion polypeptide was purified by conventional procedures.

30 EXAMPLE 2
Activity of OPG polypeptides

35 The in vivo activity of selected OPG polypeptides and OPG fusion polypeptides described in Example 1 was determined as follows. OPG preparations

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were administered by subcutaneous (SC) injection to 4-5 week old male BDF1 mice for 4 days and radiographs of the mice were taken on day 5. The positive result was for increased radiographic density in the proximal 5 tibial metaphysis compared to vehicle treated controls. There were 4 animals per group with each tibia compared to a different control tibia to give the results numbered 1-8. At least 5 of 8 results were required to be positive in order to conclude that a biological 10 response had occurred. The lowest dose giving a biological response is considered the indicator of *in vivo* potency. All doses are expressed as mg/kg/day. Daily dose experiments with truncated and full-length OPG polypeptides are shown in Table 2. Daily dose 15 experiments with OPG fusion polypeptides are shown in Table 3. OPG polypeptides and OPG fusion polypeptides having an N-terminal methionine residue were expressed in *E. coli* host cells, while those without an N-terminal methionine were expressed in CHO cells.

20

TABLE 2

Daily Dosing Experiments
X ray on day 5

25

Factor	Dose	1	2	3	4	5	6	7	8	Results
met OPG[22-194]	10.0	-	-	+	+	+	+	+	-	Positive 5/8
met OPG[22-194]	5.0	-	-	-	-	-	+	-	-	Negative 1/8
met OPG[22-194]	1.0	-	-	-	-	-	-	-	-	Negative 0/8
met OPG[22-201]	1.5	-	+	-	+	+	+	+	+	Positive 6/8
met OPG[22-201]	0.5	-	-	+	-	-	-	-	+	Negative 2/8
met OPG[22-201]	0.15	-	-	-	-	-	+	-	-	Negative 1/8
OPG[22-293]	1.5	+	+	+	+	+	+	+	+	Positive 8/8
OPG[22-293]	0.5	+	+	+	+	+	+	+	+	Positive 8/8
OPG[22-293]	0.15	-	-	-	-	-	-	-	-	Negative 0/8
OPG[22-401]	10	-	+	+	+	+	+	-	-	Positive 5/8
OPG[22-401]	4.2	-	-	-	-	-	+	+	-	Negative 2/8

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TABLE 3

Daily Dosing Experiments
X ray on day 5

5

In single dose experiments, male BDF1 mice aged 3-4 weeks received varying doses of OPG fusion proteins indicated below by a single subcutaneous injection in carrier (PBS/0.1% BSA) on day 0 (or day 1), the mice were then x-rayed on day 7 (or day 5). For each treatment, all the mice in that group and the PBS/0.1% BSA control group were x-rayed on a single film. Positive results were scored as described above. Doses are expressed in mg/Kg. The results are shown in Table 4.

20

TABLE 4

Single Dose Experiments
X ray on day 5

25

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Factor	Dose	1	2	3	4	5	6	7	8	Result
OPG[22-201]-Fc	0.3	+	+	+	+	+	+	+	+	Positive 8/8
OPG[22-201]-Fc	0.1	+	+	+	+	+	+	+	+	Positive 8/8
OPG[22-201]-Fc	0.03	+	-	+	+	-	-	-	-	Negative 3/8
OPG[22-194]-FcΔC	0.3	+	+	+	+	+	+	+	+	Positive 8/8
OPG[22-194]-FcΔC	0.1	+	+	+	+	+	+	+	+	Positive 8/8
OPG[22-194]-FcΔC	0.03	-	-	+	+	-	-	+	+	Negative 4/8

Single Dose Experiments
5 X ray on day 7

Factor	Dose	1	2	3	4	5	6	7	8	Results
met FcΔC-22-194	3.0	+	+	+	+	+	+	+	+	Positive 8/8
met FcΔC-22-194	1.0	-	-	+	+	+	-	+	+	Positive 5/8
met FcΔC-22-194	0.3	-	-	-	-	-	-	-	-	Negative 0/8
met FcΔC-22-194	0.1	-	-	-	-	-	-	-	-	Negative 0/8
OPG[22-194]-FcΔC	3.0	+	+	+	+	+	+	+	+	Positive 8/8
OPG[22-194]-FcΔC	1.0	+	+	+	+	+	+	+	+	Positive 8/8
OPG[22-194]-FcΔC	0.3	+	+	+	+	+	+	+	+	Positive 8/8
OPG[22-194]-FcΔC	0.1	-	+	-	-	+	-	+	-	Negative 3/8

It is apparent that OPG truncated polypeptides fused to an Fc region demonstrate in vivo activity at lower doses than unfused OPG truncated or full-length polypeptides. Further, OPG[22-194]-FcΔC (Fc fusion at the carboxy terminus of OPG[22-194] polypeptide) demonstrated greater in vivo potency than FcΔC-OPG[22-194] (Fc fusion at the amino terminus of OPG[22-194]).

* * *

20 While the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

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WHAT IS CLAIMED IS:

1. A protein having a formula selected from the group consisting of: R_1-R_2 , R_2-R_1 , R_1-L-R_2 , and R_2-L-R_1 , wherein R_1 is a Fc protein, or variant or fragment thereof, R_2 is an OPG protein, or variant or fragment thereof, and L is a linker.
2. The protein of Claim 1 having the formula R_2-L-R_1 .
3. The protein according to claim 1, wherein the Fc protein is selected from the group consisting of:
 - 15 (a) the Fc amino acid sequences as set forth in Figure 1;
 - (b) the amino acid sequence of subpart (a) having a different amino acid substituted or deleted in one or more of the following positions 20 (using the numbering according to Figure 1):
 - (i) one or more cysteine residues;
 - (ii) one or more tyrosine residues;
 - (iii) cysteine at position 5 deleted or substituted with an alanine;
 - (iv) leucine at position 20 deleted or substituted with glutamine;
 - (v) glutamic acid at position 103 deleted or substituted with an alanine;
 - (vi) lysine at position 105 deleted or substituted with an alanine;
 - (vii) lysine at position 107 deleted or substituted with an alanine;
 - (viii) deletion or substitution of one or more of the amino acids at positions 1, 2, 3, 4, 30 and 5;

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(ix) one or more residues substituted or deleted to ablate the Fc receptor binding site;

(x) one or more residues substituted or deleted to ablate the complement (Clq) binding site; and

5 (xi) a combination of subparts i-x;

(c) the amino acid sequence of subparts (a) or (b) having a methionyl residue at the N-terminus;

10 (d) the Fc protein, or variant, fragment or derivative thereof, of any of subparts (a) through (c) comprised of a chemical moiety connected to the protein moiety;

15 (e) a derivative of subpart (d) wherein said chemical moiety is a water soluble polymer moiety;

(f) a derivative of subpart (e) wherein said water soluble polymer moiety is polyethylene glycol; and

20 (g) a derivative of subpart (e) wherein said water soluble polymer moiety is attached at solely the N-terminus of said protein moiety.

4. The protein according to claim 1, wherein the OPG protein, or variant, fragment or derivative thereof, is selected from the group consisting of:

25 (a) the amino acid sequence 22-X wherein X is any residue from position 185 to 401 inclusive as shown in Figure 2 (SEQ ID NO: 2);

30 (b) the amino acid sequence 22-X wherein X is any residue from position 185 to 293 inclusive as shown in Figure 2 (SEQ ID NO: 2);

35 (c) the amino acid sequence of subparts (a) and (b) having a methionyl residue at the N-terminus.

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(c) the OPG protein, or variant, fragment or derivative thereof, of any of subparts (a), (b) and (c) comprised of a chemical moiety connected to the protein moiety;

5 (d) a derivative of subpart (c) wherein said chemical moiety is a water soluble polymer moiety;

(e) a derivative of subpart (d) wherein said water soluble polymer moiety is polyethylene glycol;

10 (f) A derivative of subpart (d) wherein said water soluble polymer moiety is a polyamino acid moiety; and

(g) a derivative of subpart (d) wherein said water soluble polymer moiety is attached at solely 15 the N-terminus of said protein moiety.

5. The protein of claim 1 wherein the linker is one or more amino acids selected from the group consisting of glycine, asparagine, serine, 20 threonine and alanine.

6. The protein of claim 1 wherein the linker is selected from the group consisting of:

- (a) ala-ala-ala;
- 25 (b) ala-ala-ala-ala;
- (c) ala-ala-ala-ala-ala;
- (d) gly-gly;
- (e) gly-gly-gly;
- (f) gly-gly-gly-gly-gly;
- 30 (g) gly-gly-gly-gly-gly-gly-gly;
- (h) gly-pro-gly;
- (i) gly-gly-pro-gly-gly;
- (j) val;
- (k) ser-gly-gly-gly-gly-gly-gly-

35 gly;

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(1) gly-gly-ser-gly-ser-ala-gly-ser-
gly-ser-gly-gly-gly-ser-gly-ser-gly-gly;
(m) a chemical moiety; and
(n) any combination of subparts (a)
5 through (m).

7. A fusion protein comprising the amino acid sequence selected from the group consisting of the amino acid sequences set forth in Figures 5, 6, 7 or 8
10 (SEQ ID NOS: 5, 6, 7, 8, respectively).

8. A nucleic acid sequence encoding for a protein having the formula selected from the group consisting of: R_1-R_2 , R_2-R_1 , R_1-L-R_2 , and R_2-L-R_1 wherein
15 R_1 is a Fc protein, or variant or fragment thereof, R_2 is an OPG protein, or variant or fragment thereof, and L is a linker.

9. The nucleic acid sequence of Claim 8
20 encoding for a protein comprising an Fc protein, variant, fragment or derivative portion selected from the group consisting of:

(a) the Fc amino acid sequence as set forth in Figure 1 (SEQ ID NO: 1);
25 (b) the amino acid sequence of subpart (a) having a different amino acid substituted or deleted in one or more of the following positions (using the numbering according to Figure 1):
(i) one or more cysteine residues;
30 (ii) one or more tyrosine residues;
(iii) cysteine at position 5 deleted or substituted with an alanine;
(iv) leucine at position 20 deleted or substituted with glutamine;
35 (v) glutamic acid at position 103 deleted or substituted with an alanine;

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(vi) lysine at position 105 deleted or substituted with an alanine;

(vii) lysine at position 107 deleted or substituted with an alanine;

5 (viii) deletion or substitution of one or more of the amino acids at positions 1, 2, 3, 4, and 5;

(ix) one or more residues substituted or deleted to ablate the Fc receptor binding site;

10 (x) one or more residues substituted or deleted to ablate the complement (Clq) binding site; and

(xi) a combination of subparts i-x;

15 (c) the amino acid sequence of subparts (a) or (b) having a methionyl residue at the N-terminus;

(d) the Fc protein, or variant, fragment or derivative thereof, of any of subparts (a) through (c) comprised of a chemical moiety connected to 20 the protein moiety;

(e) a derivative of subpart (d) wherein said chemical moiety is a water soluble polymer moiety;

(f) a derivative of subpart (e) wherein said water soluble polymer moiety is polyethylene 25 glycol; and

(g) a derivative of subpart (e) wherein said water soluble polymer moiety is attached at solely the N-terminus of said protein moiety.

30 10. The nucleic acid sequence according to claim 8 encoding for a protein comprising an OPG protein, variant, fragment or derivative portion selected from the group consisting of:

35 (a) the amino acid sequence 22-X wherein X is any residue from position 185 to 401 inclusive as shown in Figure 2 (SEQ ID NO: 2);

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(b) the amino acid sequence 22-X wherein X is any residue from position 185 to 293 inclusive as shown in Figure 2 (SEQ ID NO: 2);

5 (c) the amino acid sequence of subparts (a) and (b) having a methionyl residue at the N-terminus;

10 (d) the OPG protein, or variant, fragment or derivative thereof, of any of subparts (a), (b) and (c) comprised of a chemical moiety connected to the protein moiety;

(e) a derivative of subpart (d) wherein said chemical moiety is a water soluble polymer moiety;

15 (f) a derivative of subpart (e) wherein said water soluble polymer moiety is polyethylene glycol;

(g) A derivative of subpart (e) wherein said water soluble polymer moiety is a polyamino acid moiety; and

20 (h) a derivative of subpart (e) wherein said water soluble polymer moiety is attached at solely the N-terminus of said protein moiety.

11. The nucleic acid sequence of claim 8 encoding for a protein comprising a linker of one or 25 more amino acids selected from the group consisting of glycine, asparagine, serine, threonine and alanine.

12. The nucleic acid sequence of claim 8 encoding for a protein with a linker selected from the 30 group consisting of:

- (a) ala-ala-ala;
- (b) ala-ala-ala-ala;
- (c) ala-ala-ala-ala-ala;
- (d) gly-gly;
- 35 (e) gly-gly-gly;
- (f) gly-gly-gly-gly-gly;

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(g) gly-gly-gly-gly-gly-gly;
(h) gly-pro-gly;
(i) gly-gly-pro-gly-gly;
(j) val;
5 (k) ser-gly-gly-gly-gly-gly-gly-
gly;
(l) gly-gly-ser-gly-ser-gly-ala-gly-
ser-gly-ser-gly-gly-ser-gly-ser-gly-gly;
(m) a chemical moiety; and
10 (n) any combination of subparts (a)
through (m).

13. A nucleic acid sequence encoding a
fusion protein comprising the amino acid sequence
15 selecting from the group consisting of: the amino acid
sequences as set forth in Figures 5, 6, 7 or 8 (SEQ ID
NOS: 5, 6, 7, 8, respectively).

14. A vector comprising a nucleic acid
20 sequence according to any of Claims 8 to 13 inclusive.

15. A prokaryotic or eukaryotic host cell
containing the vector of claim 14.

25 16. A process for producing a protein of
claims 1 or 6 comprising the steps of culturing, under
suitable conditions, the host cell of claim 15, and
isolating the protein produced.

30 17. The process of claim 16 further
comprising the step of purifying the protein produced.

18. A pharmaceutical composition comprising
an effective amount of a protein according to claims 1
35 or 6, in a pharmaceutically acceptable diluent,
adjuvant or carrier.

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19. A method of preventing or treating a
bone loss in a mammal comprising administering a
therapeutically effective amount of the protein of any
5 of Claims 1-6.

20. The method of Claim 19 wherein the bone
loss is selected from the group consisting of
osteoporosis, Paget's disease, osteomyelitis,
10 hypercalcemia, osteopenia associated with surgery or
steroid administration, osteonecrosis, bone loss due to
rheumatoid arthritis, periodontal bone loss, osteolytic
metastasis, and prosthetic loosening.

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FIG. 1

Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala
1				5				10						15	
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro
				20				25						30	
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val
				35				40					45		
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val
				50				55				60			
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Gln	
				65				70			75			80	
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln
				85				90					95		
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala
				100				105					110		
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro
				115				120				125			
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr
				130				135				140			
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser
				145				150			155			160	
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr
				165				170				175			
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr
				180				185				190			
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe
				195				200			205				
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys
				210				215			220				
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys								
				225			230								

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FIG. 2A

Met	Asn	Lys	Trp	Leu	Cys	Cys	Ala	Leu	Leu	Val	Leu	Leu	Asp	Ile	Ile
1				5				10						15	
Glu	Trp	Thr	Thr	Gln	Glu	Thr	Leu	Pro	Pro	Lys	Tyr	Leu	His	Tyr	Asp
		20					25						30		
Pro	Glu	Thr	Gly	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Ala	Pro	Gly	Thr
			35				40						45		
Tyr	Leu	Lys	Gln	His	Cys	Thr	Val	Arg	Arg	Lys	Thr	Leu	Cys	Val	Pro
		50					55						60		
Cys	Pro	Asp	His	Ser	Tyr	Thr	Asp	Ser	Trp	His	Thr	Ser	Asp	Glu	Cys
			65				70			75			80		
Val	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu	Gln	Ser	Val	Lys	Gln	Glu
				85					90				95		
Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys	Glu	Cys	Glu	Glu	Gly	Arg	Tyr
			100				105						110		
Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys	His	Arg	Ser	Cys	Pro	Pro	Gly	Ser
			115				120						125		
Gly	Val	Val	Gln	Ala	Gly	Thr	Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Lys
			130				135						140		
Cys	Pro	Asp	Gly	Phe	Phe	Ser	Gly	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys
			145				150			155			160		
Ile	Lys	His	Thr	Asn	Cys	Ser	Thr	Phe	Gly	Leu	Leu	Leu	Ile	Gln	Lys
				165				170					175		
Gly	Asn	Ala	Thr	His	Asp	Asn	Val	Cys	Ser	Gly	Asn	Arg	Glu	Ala	Thr
				180				185					190		
Gln	Lys	Cys	Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe	Arg
				195				200					205		
Phe	Ala	Val	Pro	Thr	Lys	Ile	Ile	Pro	Asn	Trp	Leu	Ser	Val	Leu	Val
				210				215					220		

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FIG. 2B

Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
225 230 235 240

Lys Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
245 250 255

Trp Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Ile Ile Gln
260 265 270

Asp Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Leu Gly His Ser
275 280 285

Asn Leu Thr Thr Glu Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly
290 295 300

Lys Lys Ile Ser Pro Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys
305 310 315 320

Ser Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
325 330 335

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu
340 345 350

Lys Thr Ser His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr
355 360 365

Met Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu
370 375 380

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
385 390 395 400

Leu

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FIG. 3A

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
 1 5 10 15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
 20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
 35 40 45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
 50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
 65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
 85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
 100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
 115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
 130 135 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
 145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Ala Ala Ala
 165 170 175

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 180 185 190

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 195 200 205

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 210 215 220

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FIG. 3B

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
225 230 235 240

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
245 250 255

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
260 265 270

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
275 280 285

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
290 295 300

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
305 310 315 320

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
325 330 335

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
340 345 350

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
355 360 365

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
370 375 380

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
385 390 395 400

Ser Leu Ser Leu Ser Pro Gly
405

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FIG. 4A

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
 1 5 10 15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
 20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
 35 40 45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
 50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
 65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
 85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
 100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
 115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
 130 135 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
 145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile
 165 170 175

Asp Val Thr Ala Ala Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr
 180 185 190

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
 195 200 205

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
 210 215 220

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
 225 230 235 240

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FIG. 4B

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
245 250 255

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
260 265 270

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
275 280 285

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
290 295 300

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
305 310 315 320

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
325 330 335

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
340 345 350

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
355 360 365

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
370 375 380

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
385 390 395 400

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
405 410

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FIG. 5A

Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His	Tyr	Asp	Glu	Glu	Thr	Ser	His
1					5				10					15	
Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro	Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His
							20	25					30		
Cys	Thr	Ala	Lys	Trp	Lys	Thr	Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr
							35	40					45		
Tyr	Thr	Asp	Ser	Trp	His	Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro
					50		55		60						
Val	Cys	Lys	Glu	Leu	Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His
					65		70		75				80		
Asn	Arg	Val	Cys	Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe
					85			90					95		
Cys	Leu	Lys	His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala
					100			105				110			
Gly	Thr	Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe
					115			120				125			
Phe	Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
					130		135					140			
Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr	His
					145		150			155			160		
Asp	Asn	Ile	Cys	Ser	Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Val	Asp	Lys
					165			170				175			
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
					180			185				190			
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
					195			200				205			
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
					210		215				220				
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
					225		230			235			240		

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FIG. 5B

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
245 250 255

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
260 265 270

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
275 280 285

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
290 295 300

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
305 310 315 320

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
325 330 335

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
340 345 350

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
355 360 365

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
370 375 380

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
385 390 395 400

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FIG. 6A

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
 1 5 10 15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
 20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
 35 40 45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
 50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
 65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
 85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
 100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
 115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
 130 135 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
 145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile
 165 170 175

Asp Val Thr Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
 180 185 190

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 195 200 205

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 210 215 220

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 225 230 235 240

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FIG. 6B

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
245 250 255

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
260 265 270

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
275 280 285

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
290 295 300

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
305 310 315 320

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
325 330 335

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
340 345 350

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
355 360 365

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
370 375 380

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
385 390 395 400

Leu Ser Leu Ser Pro Gly
405

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FIG. 7A

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
 1 5 10 15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
 20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
 35 40 45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
 50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
 65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
 85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
 100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
 115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
 130 135 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
 145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Ser Gly Gly
 165 170 175

Gly Gly Gly Gly Gly Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
 180 185 190

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 195 200 205

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 210 215 220

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
 225 230 235 240

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FIG. 7B

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
245 250 255

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
260 265 270

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
275 280 285

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
290 295 300

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
305 310 315 320

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
325 330 335

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
340 345 350

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
355 360 365

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
370 375 380

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
385 390 395 400

Leu Ser Pro Gly

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FIG. 8A

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 1 5 10 15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 35 40 45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 65 70 75 80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 85 90 95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 100 105 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 115 120 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 165 170 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 210 215 220

Ser Pro Gly Lys Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu
 225 230 235 240

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FIG. 8B

Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr
245 250 255

Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys
260 265 270

Pro Asp His Tyr Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu
275 280 285

Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys
290 295 300

Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu
305 310 315 320

Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly
325 330 335

Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys
340 345 350

Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg
355 360 365

Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly
370 375 380

Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln
385 390 395 400

Lys

SEQUENCE LISTING

<110> Amgen Inc.

<120> OPG Fusion Protein Compositions and Methods

<130> A-604

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1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 2
<211> 401
<212> PRT
<213> Human

<400> 2
Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Leu Leu Asp Ile Ile
1 5 10 15

Glu Trp Thr Thr Gln Glu Thr Leu Pro Pro Lys Tyr Leu His Tyr Asp
20 25 30

Pro Glu Thr Gly His Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr
35 40 45

Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro
50 55 60

Cys Pro Asp His Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
65 70 75 80

Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Ser Val Lys Gln Glu
85 90 95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Gly Arg Tyr
100 105 110

Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Ser
115 120 125

Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Lys
130 135 140

Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys
145 150 155 160

Ile Lys His Thr Asn Cys Ser Thr Phe Gly Leu Leu Leu Ile Gln Lys
165 170 175

Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr
180 185 190

Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
195 200 205

Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val
210 215 220

Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
225 230 235 240

Lys Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
245 250 255

Trp Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln
 260 265 270

Asp Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Leu Gly His Ser
 275 280 285

Asn Leu Thr Thr Glu Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly
 290 295 300

Lys Lys Ile Ser Pro Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys
 305 310 315 320

Ser Ser Glu Gln Leu Leu Lys Leu Ser Leu Trp Arg Ile Lys Asn
 325 330 335

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu
 340 345 350

Lys Thr Ser His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr
 355 360 365

Met Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu
 370 375 380

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
 385 390 395 400

Leu

<210> 3
 <211> 407
 <212> PRT
 <213> Human

<400> 3
 Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
 1 5 10 15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
 20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
 35 40 45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
 50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
 65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
 85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
 100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
 115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Ala Ala Ala
165 170 175

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
180 185 190

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
195 200 205

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
210 215 220

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
225 230 235 240

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
245 250 255

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
260 265 270

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
275 280 285

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
290 295 300

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
305 310 315 320

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
325 330 335

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
340 345 350

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
355 360 365

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
370 375 380

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
385 390 395 400

Ser Leu Ser Leu Ser Pro Gly
405

<210> 4
<211> 413
<212> PRT
<213> Human

<400> 4

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
1 5 10 15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
35 40 45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile
165 170 175

Asp Val Thr Ala Ala Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr
180 185 190

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
195 200 205

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
210 215 220

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
225 230 235 240

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
245 250 255

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
260 265 270

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
275 280 285

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
290 295 300

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
305 310 315 320

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
325 330 335

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
340 345 350

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
355 360 365

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
370 375 380

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
385 390 395 400

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
405 410

<210> 5

<211> 400

<212> PRT

<213> Human

<400> 5

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
1 5 10 15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
35 40 45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Val Asp Lys
165 170 175

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
180 185 190

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 195 200 205
 Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 210 215 220
 Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 225 230 235 240
 Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
 245 250 255
 Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 260 265 270
 Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 275 280 285
 Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 290 295 300
 Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 305 310 315 320
 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 325 330 335
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 340 345 350
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 355 360 365
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 370 375 380
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 385 390 395 400

<210> 6
 <211> 406
 <212> PRT
 <213> Human

<400> 6
 Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
 1 5 10 15
 Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
 20 25 30
 Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
 35 40 45
 Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
 50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140

Cys Ser Val Phe Gly Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile
165 170 175

Asp Val Thr Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
180 185 190

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
195 200 205

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
210 215 220

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
225 230 235 240

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
245 250 255

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
260 265 270

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
275 280 285

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
290 295 300

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
305 310 315 320

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
325 330 335

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
340 345 350

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
355 360 365

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
370 375 380

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser

385 390 395 400

Leu Ser Leu Ser Pro Gly
405<210> 7
<211> 404
<212> PRT
<213> Human<400> 7
Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
1 5 10 15Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
20 25 30Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
35 40 45Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
50 55 60Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
65 70 75 80Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
85 90 95Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
100 105 110Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
115 120 125Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145 150 155 160Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Ser Gly Gly
165 170 175Gly Gly Gly Gly Gly Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
180 185 190Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
195 200 205Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
210 215 220Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
225 230 235 240Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
245 250 255

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu

10

260

265

270

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
 275 280 285

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 290 295 300

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
 305 310 315 320

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 325 330 335

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 340 345 350

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 355 360 365

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
 370 375 380

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 385 390 395 400

Leu Ser Pro Gly

<210> 8

<211> 401

<212> PRT

<213> Human

<400> 8

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 1 5 10 15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 35 40 45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 65 70 75 80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 85 90 95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 100 105 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 115 120 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val

130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
165 170 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
210 215 220

Ser Pro Gly Lys Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu
225 230 235 240

Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr
245 250 255

Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys
260 265 270

Pro Asp His Tyr Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu
275 280 285

Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys
290 295 300

Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu
305 310 315 320

Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly
325 330 335

Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys
340 345 350

Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg
355 360 365

Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly
370 375 380

Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln
385 390 395 400

Lys

<210> 9
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

Oligonucleotide

<400> 9
aagtctagac caccatgaac aagttgctgt 30

<210> 10
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 10
gctagtcgac tactcgaagg tgaggtagc at 32

<210> 11
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 11
atctgtcgac tatttttagt ttgattcac 29

<210> 12
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 12
aagtctagac caccatgaac aagttgctgt 30

<210> 13
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 13
cacgcgtcga ctttttgagt tgattcactg tttcc 35

<210> 14
<211> 26
<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 14

aatctgtcga caaaaactcac acatgc

26

<210> 15

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 15

ccatgctcga gttatcattt acccggagac agg

33

<210> 16

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 16

aatccggagg aggtgggtgga ggtgggggta cctgcccacc gtgc

44

<210> 17

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 17

ccatgctcga gttatcattt acccggagac agg

33

<210> 18

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 18

aagtctagac caccatgaac aagttgctgt

30

<210> 19
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 19
cctccggatt tttgagttga ttcactgttt ccaga

35

<210> 20
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 20
ttggcgcc caaatcttgt gacaaaact

29

<210> 21
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 21
cttggagga aacgtttctt tacccggaga caggga

36

<210> 22
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 22
tccctgtctc cgggtaaaga aacgtttctt ccaaag

36

<210> 23
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 23
atctgtcgac tattttgag ttgattcac 29

<210> 24
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 24
aacaaactct agatttgttt taactaatta aagg 34

<210> 25
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 25
aggaataaca tatggaaaact ttccaccta aatatcttca ttatgtatgaa 50

<210> 26
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 26
gaaactagtc accagctgct gtgcgacaaa tgcctccgg gtacctacct 50

<210> 27
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 27
gaaacagcac tgcaccgcta aatggaaaac cgtttgcgt ccttgcgg 50

<210> 28
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 28
accactacta caccgactcc tggcacacacct ccgacgaatg cctgtactgc 50

<210> 29
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 29
tcaccggttt gcaaggagct gcagtacgtt aaacaggaat gcaaccgtac 50

<210> 30
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 30
gcacaaccgt gtttgcgaat gcaaagaagg tcgttacctg gagatcgaat 50

<210> 31
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 31
tctgcctgaa acaccgttcc tgtccgcctg gtttcgggtgt tgtacaggct 50

<210> 32
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 32
ggtaccccccgg aacgtaacac cgtttgcaaa cgttgcccccgg acggtttctt 50

<210> 33
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 33
ctccaaacgaa acctcgagca aagctccgtg ccgtaaacac accaactgct 50

<210> 34
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 34
ccgttttcgg ttcctgtta acccagaaaag gtaacgctac ccacgacaac 50

<210> 35
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 35
atctgctccg gtaactccga gtcgacccag aaataatgga tcccaaacaa 50

<210> 36
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 36
ttgtttggga tccattatcc ctgggtcgac tcgg 34

<210> 37
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 37
agttagccga gcagatgttgcgtggtag cgttacctt ctgggttaac 50

<210> 38
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 38
aggagaccga aaacggagca gttgggtgtgt ttacggcacg gagctttgct 50

<210> 39
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 39
cgaggtttcg ttggagaaga aaccgtccgg gcaacgtttg caaacggtgt 50

<210> 40
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 40
tacgttccgg ggtaccagcc tgtacaacac cgaaaccagg cggacaggaa 50

<210> 41
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 41
cggtgtttca ggcagaattt gatctccagg taacgacctt ctttgcattt 50

<210> 42
<211> 50
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 42

gcaaacacagg ttgtgcgtac ggttgcattc ctgtttaacg tactgcagct

50

<210> 43

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 43

ccttgcaaac cggtgagcag tacaggcatt cgtcggaggt gtgccaggag

50

<210> 44

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 44

tcggtgtagt agtgtccgg acaaggagcg caaacggttt tccatTTAGC

50

<210> 45

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 45

ggtgcaGTgc tgTTTCAGGT aggtacCCGG aggacatTTG tcgcacAGCA

50

<210> 46

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 46

gctgggtgact agTTTCTTCA tcataatgaa gatatttagg tggaaaAGTT

50

<210> 47

<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 47
tccatatgtt attcctcctt taatttagtta aaacaaatct agagtttgtt 50

<210> 48
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 48
cgtacagggtt tacgcaagaa aatgg 25

<210> 49
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 49
acaaacacta gtttcttcat cataatgaag atattttagt ggaaacgt 48

<210> 50
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 50
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SEQUENCE LISTING

<110> Wooden, Scott K.
Mann, Michael B.
Dunstan, Colin R.

<120> OPG Fusion Protein Compositions and Methods

<130> A-604

<140>

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<160> 50

<170> PatentIn Ver. 2.1

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<211> 232

<212> PRT

<213> Human

<400> 1

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35 40 45Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145 150 155 160Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220Ser Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 2

<211> 401

<212> PRT
<213> Human

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Pro Glu Thr Gly His Gln Leu Leu Cys Asp Lys Cys Ala Pro Gly Thr
35 40 45
Tyr Leu Lys Gln His Cys Thr Val Arg Arg Lys Thr Leu Cys Val Pro
50 55 60
Cys Pro Asp His Ser Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys
65 70 75 80
Val Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Ser Val Lys Gln Glu
85 90 95
Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Glu Glu Gly Arg Tyr
100 105 110
Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Ser
115 120 125
Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Lys
130 135 140
Cys Pro Asp Gly Phe Phe Ser Gly Glu Thr Ser Ser Lys Ala Pro Cys
145 150 155 160
Ile Lys His Thr Asn Cys Ser Thr Phe Gly Leu Leu Leu Ile Gln Lys
165 170 175
Gly Asn Ala Thr His Asp Asn Val Cys Ser Gly Asn Arg Glu Ala Thr
180 185 190
Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
195 200 205
Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val
210 215 220
Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
225 230 235 240
Lys Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
245 250 255
Trp Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln
260 265 270
Asp Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Leu Gly His Ser
275 280 285
Asn Leu Thr Thr Glu Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly
290 295 300

Lys Lys Ile Ser Pro Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys
305 310 315 320

Ser Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
325 330 335

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu
340 345 350

Lys Thr Ser His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr
355 360 365

Met Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu
370 375 380

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
385 390 395 400

Leu

<210> 3
<211> 407
<212> PRT
<213> Human

<400> 3
Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
1 5 10 15

Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
35 40 45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Ala Ala Ala
165 170 175

24

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 180 185 190

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 195 200 205

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 210 215 220

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
 225 230 235 240

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 245 250 255

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
 260 265 270

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
 275 280 285

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
 290 295 300

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
 305 310 315 320

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
 325 330 335

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
 340 345 350

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
 355 360 365

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
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Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
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Ser Leu Ser Leu Ser Pro Gly
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<210> 4

<211> 413

<212> PRT

<213> Human

<400> 4

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Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
 20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
 35 40 45

25

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
115 120 125

Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140

Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145 150 155 160

Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile
165 170 175

Asp Val Thr Ala Ala Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr
180 185 190

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
195 200 205

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
210 215 220

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
225 230 235 240

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
245 250 255

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
260 265 270

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
275 280 285

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
290 295 300

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
305 310 315 320

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
325 330 335

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
340 345 350

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
355 360 365

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp

26

370 375 380

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
385 390 395 400Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
405 410

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<211> 400

<212> PRT

<213> Human

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Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
1 5 10 15Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
20 25 30Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
35 40 45Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
50 55 60Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
65 70 75 80Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
85 90 95Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
100 105 110Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
115 120 125Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145 150 155 160Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Val Asp Lys
165 170 175Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
180 185 190Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
195 200 205Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
210 215 220Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
225 230 235 240

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val

27

245

250

255

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 260 265 270

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 275 280 285

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 290 295 300

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 305 310 315 320

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 325 330 335

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 340 345 350

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 355 360 365

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 370 375 380

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 385 390 395 400

<210> 6

<211> 406

<212> PRT

<213> Human

<400> 6

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His
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Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
 20 25 30

Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
 35 40 45

Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
 50 55 60

Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
 65 70 75 80

Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
 85 90 95

Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
 100 105 110

Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe

115	120	125
Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr His		
145	150	155
160		
Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile		
165	170	175
Asp Val Thr Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro		
180	185	190
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys		
195	200	205
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val		
210	215	220
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp		
225	230	235
240		
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr		
245	250	255
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp		
260	265	270
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu		
275	280	285
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg		
290	295	300
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys		
305	310	315
320		
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp		
325	330	335
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys		
340	345	350
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser		
355	360	365
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser		
370	375	380
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser		
385	390	395
400		
Leu Ser Leu Ser Pro Gly		
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<212> PRT
<213> Human

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Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His
20 25 30
Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr
35 40 45
Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro
50 55 60
Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His
65 70 75 80
Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe
85 90 95
Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala
100 105 110
Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe
115 120 125
Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn
130 135 140
Cys Ser Val Phe Gly Leu Leu Thr Gln Lys Gly Asn Ala Thr His
145 150 155 160
Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Ser Gly Gly
165 170 175
Gly Gly Gly Gly Gly Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
180 185 190
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
195 200 205
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
210 215 220
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
225 230 235 240
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
245 250 255
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
260 265 270
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
275 280 285
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
290 295 300
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
305 310 315 320

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
325 330 335

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
340 345 350

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
355 360 365

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
370 375 380

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
385 390 395 400

Leu Ser Pro Gly

<210> 8

<211> 401

<212> PRT

<213> Human

<400> 8

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20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
35 40 45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65 70 75 80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
85 90 95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
100 105 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
115 120 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
165 170 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
210 215 220

Ser Pro Gly Lys Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu
225 230 235 240

Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr Tyr
245 250 255

Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro Cys
260 265 270

Pro Asp His Tyr Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys Leu
275 280 285

Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu Cys
290 295 300

Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu
305 310 315 320

Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly
325 330 335

Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys
340 345 350

Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg
355 360 365

Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly
370 375 380

Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln
385 390 395 400

Lys

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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

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30

<210> 10
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

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32

<210> 11
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 11
atctgtcgac tattttgag ttgattcac

29

<210> 12
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 12
aagtctagac caccatgaac aagttgctgt

30

<210> 13
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 13
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35

<210> 14
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 14
aatctgtcga caaaaactcac acatgc

26

<210> 15
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 15
ccatgctcga gttatcattt acccggagac agg

33

<210> 16
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 16
aatccggagg aggtgggtgga ggtgggggta cctgcccacc gtgc

44

<210> 17
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 17
ccatgctcga gttatcattt acccggagac agg

33

<210> 18
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 18
aagtctagac caccatgaac aagttgctgt

30

<210> 19
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 19
cctccggatt tttgagttga ttcactgttt ccaga

35

<210> 20
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 20
ttggcgcgcc caaatcttgt gacaaaact

29

<210> 21
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 21
ctttggagga aacgtttctt taccggaga caggga

36

<210> 22
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 22
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36

<210> 23
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 23
atctgtcgac tatttttgag ttgattcac

29

<210> 24
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 24
aacaactct agattgttt taactaatta aagg

34

<210> 25
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 25
aggaataaca tatggaaaact tttccaccta aatatcttca ttatgtatgaa

50

<210> 26
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 26
gaaactagtc accagctgct gtgcgacaaa tgcctccgg gtacacct

50

<210> 27
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 27
gaaacagcac tgcaccgcta aatggaaaac cgtttgcgct cttgtccgg

50

<210> 28
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 28
accactacta caccgactcc tggcacaccc tccgacgaatg cctgtactgc

50

<210> 29

<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 29
tcaccggttt gcaaggagct gcagtacgtt aaacaggaat gcaaccgtac 50

<210> 30
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 30
gcacaaccgt gtttgcgaat gcaaagaagg tcgttacctg gagatcgaat 50

<210> 31
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 31
tctgcctgaa acaccgttcc tgtccgcctg gtttcggtgt tgtacaggct 50

<210> 32
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 32
ggtaccccg aacgtaacac cgttgcaaa cgttgcccgg acggtttctt 50

<210> 33
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 33

ctccaaacgaa acctcgagca aagctccgtg ccgtaaacac accaactgct 50

<210> 34
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 34
ccgtttcgg ttcctgtta acccagaaaag gtaacgctac ccacgacaac 50

<210> 35
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 35
atctgctccg gtaactccga gtcgacccag aaataatgga tcccaaacaa 50

<210> 36
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 36
ttgtttggga tccattatcc ctgggtcgac tcgg 34

<210> 37
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 37
agttaaccgga gcagatgttgcgtggtag cgttaccctt ctgggttaac 50

<210> 38
<211> 50
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 38

aggagaccga aaacggagca gttggtgtgt ttacggcacg gagcttgct

50

<210> 39

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 39

cgagggttcg ttggagaaga aaccgtccgg gcaacgtttg caaacggtgt

50

<210> 40

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 40

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50

<210> 41

<211> 50

<212> DNA

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<400> 41

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<210> 42

<211> 50

<212> DNA

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<223> Description of Artificial Sequence: Synthetic Oligonucleotide

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<210> 43

<211> 50

<212> DNA
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<223> Description of Artificial Sequence: Synthetic Oligonucleotide

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<210> 44
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

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<210> 45
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 45
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<210> 46
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 46
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<210> 47
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Oligonucleotide

<400> 47
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<210> 48
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 48
cgtacaggtt tacgcaagaa aatgg

25

<210> 49
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

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48

<210> 50
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
Oligonucleotide

<400> 50
gaagatatTTT aggtggaaac gtttctttac ccggagacag ggag

44

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INTERNATIONAL SEARCH REPORT

Inten. Application No.
PC1/US 00/22797A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/715 C12N15/62 C07K19/00 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 196 54 610 A (AMGEN INC) 26 June 1997 (1997-06-26) cited in the application page 7, line 51 - line 57	1, 3, 4, 7-10, 13-20
Y	page 9, line 21 - line 45 ---	2, 5, 6, 11, 12
Y	US 5 457 035 A (BAUM PETER R ET AL) 10 October 1995 (1995-10-10) column 6, line 10 - line 14 column 6, line 32 - line 37 column 6, line 63 -column 7, line 32 ---	2, 5, 6, 11, 12
		-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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Date of the actual completion of the International search

19 January 2001

Date of mailing of the International search report

26/01/2001

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INTERNATIONAL SEARCH REPORT

Inten of Application No
PCT/US 00/22797

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	MORONY S. ET AL.: "A chimeric form of osteoprotegerin inhibits hypercalcemia and bone resorption induced by IL-1beta, TNF-alpha, PTH, PTHrP, and 1,25(OH)2D3." JOURNAL OF BONE AND MINERAL RESEARCH, vol. 14, no. 9, 1999, pages 1478-1485, XP000971493 ISSN: 0884-0431 the whole document -----	1,3,4, 7-10, 13-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/22797

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